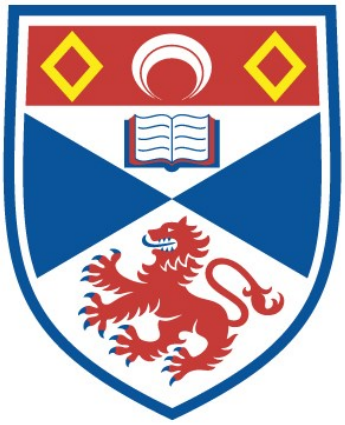
STUDIES ON THE GROWTH AND PHYSIOLOGY OF ATTACHED MARINE ALGAE

Barry Paul Jupp

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1972

Studies on the growth and physiology of attached marine algae

by

Barry Paul Jupp

A thesis submitted after nine terms of study, from October 1969 to December 1971, under Ordinance General No. 12, for the degree of Doctor of Philosophy.

March, 1972

Gatty Marine Laboratory,
and Department of Botany,
University of St. Andrews.

Laminaria hyperborea at Extreme Low Water Spring Tide

DECLARATION

I hereby declare that the following thesis is based upon work carried out by me and that it has not been previously submitted for a higher degree, and that it is my own composition.

The studies were carried out under the supervision of Dr. E.A.Drew, Gatty Marine Laboratory, St. Andrews.

CERTIFICATE

I certify that Barry P. Jupp has spent nine terms of research under my direction, that he has fulfilled the conditions of Ordinance General No. 12 and Resolution of the University Court 1967, No. 1, and that he is qualified to submit the accompanying thesis in application for the degree of Doctor of Philosophy.

Signed

Dr. E.A.Drew,
Gatty Marine Laboratory,
University of St. Andrews.

Curriculum vitae

I was born in Corsham, Wiltshire, in 1945, and was educated at Dursley Grammar School, Chipping Sodbury Grammar School, Hull University and Reading University. I graduated with a Second Class (Division II) Honours degree in Botany at the University of Hull in 1967. I was awarded the degree of MSc in the Technology of Crop Protection at the University of Reading in 1968. Since October 1968 I have held a N.E.R.C. Research Assistantship during which time the studies for this thesis were carried out.

Acknowledgements

I would like to express my sincere gratitude to Dr. E.A.Drew for his advice, supervision and assistance during these studies.

I would also like to thank Dr. D.C.Weeks for helpful advice, Dr. L.V.Evans for preparing the electron micrographs, and Dr. J.M.Kain (Mrs. N.S.Jones) for supplying sporophyte cultures.

I wish to thank technical staff at the Gatty Marine Laboratory and Department of Botany, University of St. Andrews. I am very grateful to numerous people in the University of St. Andrews Sub-Aqua Club for assistance in often arduous conditions during field work. My thanks also go to my parents for their support.

This research was carried out during the tenure of a N.E.R.C. Research Assistantship of 3½ years duration under Grant GR/3/484. I would also like to thank Miss F. Davidson and Mr. P. Adamson, Computing Laboratory, University of St. Andrews for assistance with biometric analysis.

CONTENTS

CHAPTER 1
INTRODUCTION

A great deal of information has accumulated on the ecology of littoral algae (see Lewis, 1964), but much less data is available for species in the more inaccessible sublittoral region. The commercial importance of the sublittoral kelps and rockweeds as a source of chemicals (especially alginic acid) led to extensive surveys of available supplies for industry (see Chapman, 1970 for a review). Thus the Scottish Seaweed Research Association was formed in 1944 mainly to carry out quantitative surveys around the Scottish coasts of sublittoral seaweeds in particular, as well as a research and development programme on various aspects of chemistry, processing and application (S.S.R.A. Annual Reports, 1946-1968).

Chapman (1944) points out the difficulties for the surface surveyor of the completely submerged species such as Laminaria hyperborea. These studies, using grabs, dredges, and view boxes, gave a detailed assessment of cover, density, and quantities of seaweed but, as Baardseth (1954) points out, the spring grab collects only 30% to 65% of its sampling capacity so that standing crop estimates from this work are not very reliable.

Scagel (1966) has emphasised the importance of S.C.U.B.A. studies and the advent of direct observation and sampling in the sublittoral with the introduction of diving techniques has resulted in more detailed ecological work. Table 1 lists the main workers in this field and the progression from descriptive studies to ecological studies is evident.

Table. 1.

The development of sublittoral ecology.

Author	Date	Equipment	Type of Study
Gislén	1930	Surface demand	Phytosociology,
Kitching et al.	1934	"	Descriptive
Kitching	1937; 1941	"	Ecology
Kornás & Kornás	1948	"	Descriptive, phytosociology
Drach	1948	SCUBA	Descriptive
Pérès & Picard	1949	"	Descriptive
Forster	1954;1955;1958	"	Descriptive

Author	Date	Equipment	Type of Study
Knight-Jones & Jones	1956	SCUBA	Descriptive
Aleem	1956	"	Ecology
Burrows	1956;1958	"	Ecology
North	1958;1961;1964	"	Ecology
Gilmartin	1960	"	Ecology
Kain	1960-1971	"	Descriptive, ecology,
Neushul	1958;1961	"	Ecology
Neushul & Haxo	1963	"	Culture studies, ecology,
Goreau	1963	"	Productivity
Grossett et al.	1965;1966	"	Ecology
Drew	1966	"	Productivity
Leighton et al.	1966	"	Ecology
Zaneveld	1966	"	Descriptive Ecology
Bellamy et al.	1967a,b,c; 1968, a,b,c	"	Pollution
Larkum et al	1967;1968	"	Ecology
Drew & Larkum	1967	"	Productivity
Drew	1969b	"	Productivity
Lüning	1969,a,b;1970,a,b;1971,a,b	"	Descriptive, Ecology
Norton & Burrows	1969,a,b.	"	Culture studies, Ecology
Johnston	1969	"	Productivity
Drew	1972a	"	Productivity
Drew et al.	1972,a,b.	"	Productivity
Larkum	1972	"	Ecology

Thus, the emphasis in recent studies of sublittoral ecology has been on long term production studies and dynamic relationships in the ecosystem. They have been concerned with production rates, interactions with consumers, the energy flow in the ecosystems, and the importance of key environmental factors on these dynamic processes.

The study described in this thesis has attempted to analyse the interaction between physiological processes and several environmental factors in the dominant sublittoral alga Laminaria hyperborea (Gunn.) Foslie. As Odum (1959) emphasised, experimental ecology, in attempting to correlate limiting factors in the environment with the performance of an organism,

should involve laboratory experimentation, field observation, and field experimentation. The inshore marine environment is complex and Bellamy & Whittick (1968a) have outlined the various factors which operate in and on the kelp forest ecosystem. In any autecological study it is generally held that final proof of a causal relationship between a particular environmental factor and plant performance can only be obtained in carefully controlled experiments in which all other factors except that under investigation are held constant; however, as Daubenmire (1959) and Grieg-Smith, (1964) point out, since this is a very unnatural situation for the plant and that factors in the environmental complex probably interact with each other to extract a given response in nature, the value of the extrapolation of such data to the growth of the plant in the natural environment is dubious. Blackman & Rutter (1950) have emphasised the importance of multifactorial experiments and precise field studies in the analysis of 'key' or limiting factors in the environmental control of plant growth.

The ideal instrument with which to analyse the effect of the environment on plant growth is the plant itself and this 'phytometer' approach has been adopted successfully in studies of the effect of pollutants, light, temperature, depth, grazing, and exposure on the performance of kelp forest ecosystems by Bellamy et al. (1967a,b,c); Bellamy & Whittick (1968a,b); John (1968, 1969); Whittick (1969). The performance of the plant, measured in those studies as net annual primary production obtained from biomass increment cropping, is a useful indication of the total environmental complex acting on the plant in a particular habitat; meaningful site comparisons, however, require accurate measurement of as many environmental factors as possible and then preferably the thin shell of environment immediately adjacent to the plant tissue. However, as Bellamy & Whittick (1968a) point out, complex data logging equipment can very easily be lost or damaged underwater and one of the main aspects of this work has been to develop an experimental system for measuring photosynthesis and light

intensity under as near natural conditions as possible.

The growth of a plant is a result of the interaction between the complex of environmental factors and the metabolism of the plant, and the resulting net production may be measured in various ways. Westlake (1963) gives a comprehensive review of all aspects of plant productivity and the two main methods for determining annual productivity are critically assessed in that review. These are the 'harvest' or increment cropping method (Odum, 1959; Penfound, 1956) and the measurement of the rate of photosynthesis. Biomass increment cropping is very useful in plant communities that show a marked annual fluctuation of biomass and have few losses up to the seasonal maximum biomass; this technique has been used in the present work to measure net annual primary production in Laminaria hyperborea. Shortterm photosynthesis rates have also been measured and these, combined with controlled studies in the laboratory, give some indication of the ability of the plant to grow under varying environmental conditions. Of particular interest has been the ability of the alga to grow at various depths and at various times during the growing season.

The rate of photosynthesis gives an estimate of the performance of the plant and together with a knowledge of the rates of losses via respiration, grazing, sporogeresis, exudation etc., a budget of energy flow through the plant can be made. Autotrophic plants ultimately rely on the efficiency with which total solar energy is trapped and converted into organic materials. The net productivity achieved depends on the total solar energy available and those factors in the environment which affect the rate of photosynthesis. Rabinowitch (1956) outlines many of these factors, and Thomas (1955) and Talling (1961) discuss photosynthesis under natural conditions.

The growth and productivity of littoral and sublittoral attached marine algae has been studied under field conditions by Gail (1922); Tschudy (1934); Printz (1939); Tikhovskaya (1940) and Levring (1947,1966,1967)

using the Winkler oxygen technique developed by Gaarder & Gran (1927). Sargent & Lantrip (1952) measured the photosynthesis 'in situ' of various regions of Macrocystis pyrifera and from the data inferred that translocation of organic matter into the growing tips was necessary to support growth. Black (1949) and Black & Dewar (1949), using pH and oxygen levels as a measure of photosynthesis, attempted to correlate the seasonal growth of three Laminaria species with physical and chemical factors in the environment. Blinks (1955) and Kanwisher (1966) have also measured photosynthesis of attached marine macrophytes under natural conditions. More recently the C^{14} method for measuring photosynthesis 'in situ', introduced by Steeman Nielsen (1952) has been used to study rates of primary production in macrophytic algae.

Other parameters of growth such as stipe length, lamina length and expansion, biomass changes with depth and season, and the growth of stages in the life cycle under controlled conditions, have been correlated with ecological factors by Parke (1948b); Black (1950 a,b); Co nover (1958); MacFarland & Prescott (1959); Sundene (1962, 1964); Neushul & Haxo (1963); Kain (1963-71); Bellamy & Whittick (1968a); Norton & Burrows (1969a,b) and Lüning (1969,a,b; 1970a).

Previous work has outlined the importance of light intensity (Kain, 1966; Levring, 1966; John, 1968; Whittick, 1969; Drew et al. 1972a,b) and temperature (Kniep, 1914; Harder, 1915; Ehrke, 1931; Sundene, 1962, 1964; Tseng et al., 1957; Kanwisher, 1966; Newell & Pye, 1968) whilst others have emphasised the importance of interactions between the two factors and their combined effect on photosynthesis and respiration (Tikhovskaya, 1940; Levring, 1966).

The studies in this thesis have been concerned with analysing the effect of some environmental factors on physiological processes in Laminaria hyperborea and any adaptations that exist in the alga to overcome adverse conditions.

The Species; Their Ecosystem and Distribution

A brief résumé follows on the facts known of the life cycle, ecosystem, and distribution of the sublittoral species studied in this thesis.

The Species.

Laminaria hyperborea (Gunn.) Foslie

Details of the early development, structure, habit, and reproduction of this alga are to be found in Fritsch (1945). Kain (1964, 1965) has investigated the light and temperature requirements of the gametophyte and early sporophyte stages of this alga in the laboratory. It is clear that spores are well adapted to survive long periods at the very low ambient light intensity underwater during the period of sporogenesis (October to March). The minimum irradiance for growth and the light saturation levels found for gametophytes and early sporophytes (1-2 μg cal./cm^2 sec and 50-60 μg cal./cm^2 sec respectively , Kain, 1966,1969) are particularly low compared with other species such as Macrocystis.

Successful gametophyte development is restricted to stable substrates such as large boulders or continuous rock (Walker, 1947; Kain, 1962). The mode of growth of the sporophyte has been described by Kain (1963, 1966) but the important feature is the localised primary growing region at the transition zone between the lamina and the stipe. The primary growth (increase in length of stipe and production of a new lamina) is fast from January to June and slow for the rest of the year. Secondary growth is achieved by a subsurface meristem in the outer cortex of the stipe; fast secondary growth results in an increase of the girth of the stipe. The cortical cells produced during this fast secondary growth are large and produce regions easily distinguished with the unaided eye from those composed of the small opaque cells produced during the slow period of secondary growth, thus producing annual rings. It is possible to age the plant by taking sections of the stipe near the holdfast and counting the

annual growth rings (Black et al., 1959).

The holdfast and stipe are perennial and plants up to 13 years have been found, with longevity increasing in more northerly latitudes (Kain, 1963, 1967). More haptera whorls appear in the first two to three years of growth than in subsequent years and growth of these is also seasonal (Kain, 1963). Thus the mature plant consists of a complex holdfast region and a rigid stipe which can tolerate all but the fiercest storms; both parts are often densely covered with epiphytic red algae and the holdfast in particular may be encrusted with tube worms, polyzoans etc. (Kitching 1941). The terminal lamina is often covered with hydroids and polyzoans and the effect of these epibioses on the photosynthetic capacity of the lamina and stipe has been investigated in the present study.

Individual plants may reach a length of 2m (Kitching, 1941) and there is a general tendency for greater productivity farther north. Thus Larkum (1972) quotes maximum stipe lengths of 55cm in Cornwall and 120cm in South West Ireland, whilst the maximum stipe length recorded in the present study was 180cm (West Scotland) and Spence (1918) found 2m stipes in Orkney. Stipe weight is a better index of plant growth than stipe length as it shows a long term response to the environment (Kain, 1966; Whittick, 1969). On this basis Bellamy & Whittick (1968a) suggested the most productive site in a comparative survey around British coasts was just north of Aberdeen, whilst Kain (1967) stated that the latitude of southern Norway is at or near the optimum for establishment and attainment of large size in this species. Larkum (1972) quoted maximum lamina areas of 20 dm^2 at Cornwall (March), 40 dm^2 at South West Ireland (July) and Lüning (1969b) quoted areas of 15 dm^2 in Helgoland where growing conditions for L. hyperborea are poor. Drew (1971) quoted areas of 100 dm^2 for populations in the Shetlands. These geographical differences may be the result of climatic changes and the increased longevity in northern plants already mentioned.

The vertical distribution of L. hyperborea is from E.L.W.S. (extreme low water of spring tides), where it can form a mixed forest with L. digitata, to depths of 37m in very clear water (Whittick, 1969), although the usual depth limit around Scotland is 15 to 20m, (Kain, 1962).

L. hyperborea is the dominant member of the sublittoral kelp forest ecosystem (Kitching, 1941; Kain, 1962) and forms an extensive forest restricted to solid rock. The density of plants may decrease at the lower limit (Walker, 1947) giving rise to what Kitching (1941) has described as the 'park' region. Competition with other macrophytic brown algae has been investigated by Kain (1966, 1969).

The structure of the L. hyperborea forest consists of several layers (after Kitching et al., 1934 and Whittick, 1969)

(i) An encrusting layer of mainly red algal lithophytes on the bed rock.

(ii) A ground or understorey layer of small macrophytes.

(iii) A canopy layer of the dominant.

(iv) An often abundant epiphytic element on stipes and holdfasts.

(v) An epizoic element, especially on the holdfasts, and sometimes on the lamina.

The sporophytes of the dominant species L. hyperborea are present as both canopy and understorey plants.

Some of the factors concerned with both the lower limit of growth of this species and also the seasonal growth of this alga have been investigated in the present study.

Laminaria saccharina (L.) Lamour.

The growth, reproduction, and longevity of this species has been investigated fully by Parke (1948). The main feature of this plant pertainable to this study is that it is found as a dominant sublittoral alga on solid rock in sheltered waters and can grow on much less stable substrates than L. hyperborea (Kain, 1962). The flexible stipe means it does not form an extensive canopy but trails on the substrate.

Saccorhiza polyschides (Lightf.) Batt.

Details of the life history, seasonal growth, and distribution of this species are given by Norton & Burrows (1969 a,b). The light requirements of the early sporophyte has been determined by Norton & Burrows (loc cit) and Kain (1969). The most important feature to be noted here is that this species is a fast growing annual or biennial species and is considered an 'opportunist' alga which can grow rapidly in any space available amongst the more long-lived L. hyperborea populations (Norton & Burrows, 1969a).

CHAPTER 2

MATERIALS AND METHODS

Contents

A. MATERIALS

1. Sources and preparation of algae

Quadrat samples were collected from Arisaig, Inverness-shire (56°57'N; 05°52'W) and one collection from each of the following localities; St. Abbs (55°55'N;02°08'W), Fife Ness (56°17'N;02°36'W), and Oban (56°25'N; 05°28'W).

The material used in 'in situ' experiments was that found at the various sites as described in Chapter 5 . Samples of L.hyperborea used in laboratory experiments were obtained from either St. Andrews Bay or Fife Ness. These specimens were maintained in constant flowing seawater tanks at ambient sea temperature (± 1°C) in dim light. Epiphyte free tissues were always used except in one 'in situ' experiment.

2. Reagents

'Analar' Biochemical or Laboratory Reagent chemicals from British Drug Houses, Poole were used in most cases. The collection of vitamins used in the cultures were kindly provided by Dr. G. Goodlad (Dept. of Biochemistry, University of St. Andrews).

Radioactive chemicals were obtained from the Radiochemical Centre, Amersham. Labelled compounds used were sodium bicarbonate -Cl^4 and D-glucose-U-Cl^4.

B. METHODS

Experimental procedures

1. Collection and treatment of quadrat samples

Most collections of quadrats were made from an inflatable dinghy, but on one occasion a collection was made from the shore. A 0.5 square metre quadrat was used, constructed of ½ inch square mild steel with an internal side of 70.7cm. Adjustable buoyancy life-jackets (ABLJ's) were used in transporting the quadrat and the kelp samples. The operator swam over the kelp forest and, at each sampling depth, subjectively selected representative

stands of L. hyperborea, since the study was concerned with overall causal factors determing production both at different depths and over the season, and not with patterns of variation therein.

All the plants whose holdfasts were inside this area were collected by gently prising each holdfast off the substrate with a diver's knife or crowbar. Care was taken not to damage the haptera. All the plants were either gathered together and tied with string, or put into sacks for recovery. The water depth at the level of the holdfasts was measured with a Bourdon tube depth gauge and, after correcting for the height of the tide, were expressed as metres below mean low water springs (M.B.L.W.S.).

The samples were transported back to St. Andrews (for Arisaig samples a journey of six hours), either in tanks of sea-water or wrapped in moistened sacks; no appreciable dessication occurred as weighings in the laboratory at St. Andrews showed no significant difference to weighings made on site on one occasion. The samples were stored in a cold room at 2°C until analysed (normally the next day).

2. The 'in situ' carbon-14 technique

The details of this technique are also given in Drew (1966), Drew & Larkum (1967), and the slightly modified version of the method for use in British waters is described by Drew et al. (1972b) and Drew (1972a).

The main feature of the technique is that short term photo-synthesis rates may be measured for various algal tissues under as near natural conditions as possible.

Outline of method. The primary production of algae such as Laminaria hyperborea, was measured by the 'in situ' incubation of tissues of the alga in sealed glass jars containing seawater in which theinorganic carbon pool had been labelled with carbon-14 labelled sodium bicarbonate. The uptake of carbon-14 was taken as a measure of the photosynthetic capacity of the tissue.

Apparatus and equipment

The incubation platforms (1) shown in Figure 2:1 were buoyed

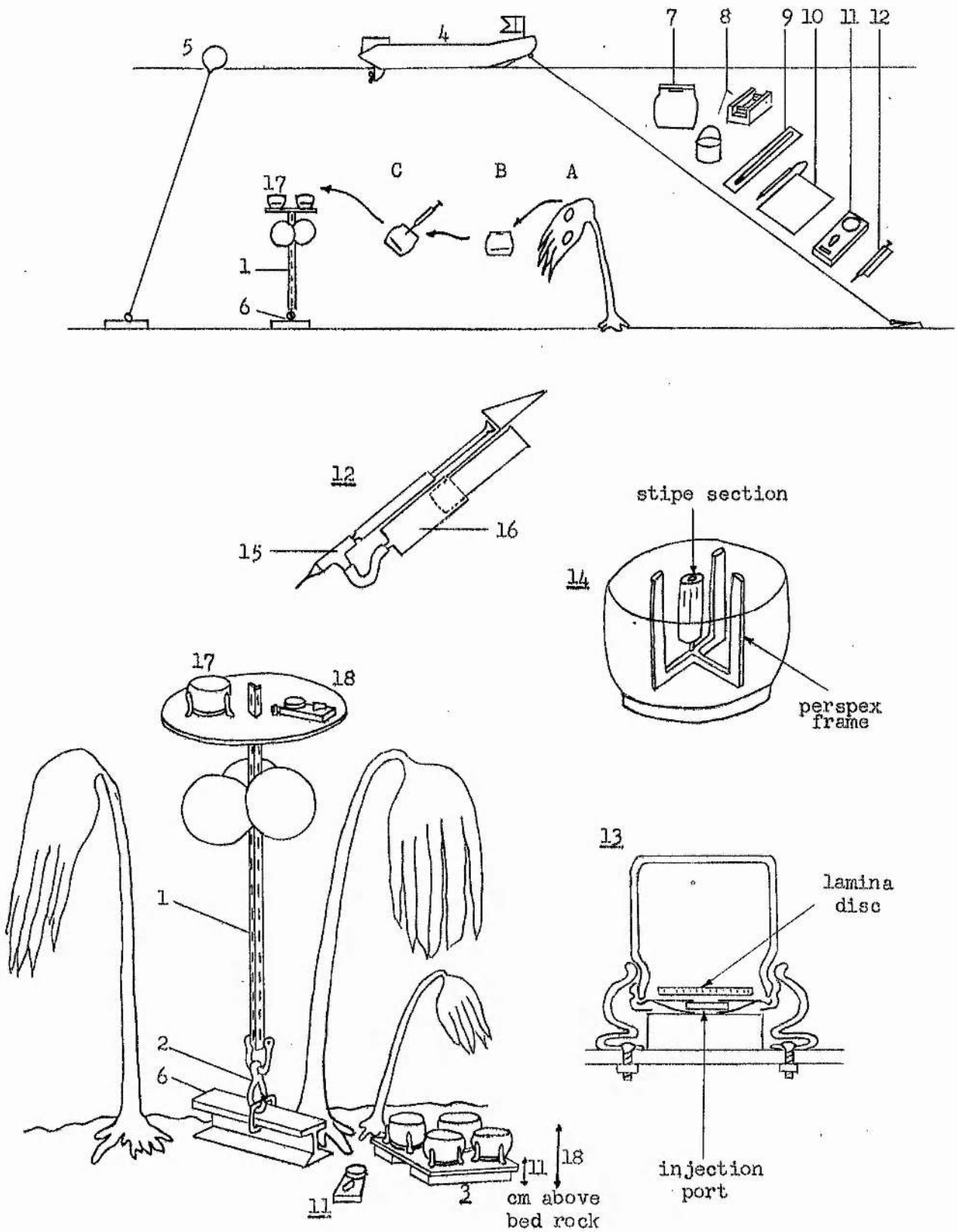


Figure 2:1 Diagrammatic representation of apparatus and procedures carried out in C14 'in situ' experiments

up from heavy bases (6) so that the incubation jars (7) clamped on top experienced some movements from water currents, thus aiding mixing inside the jars. These platforms could withstand considerable turbulence, were easily set in place and removed by spring shackles (2) at the base. The Terry clips on top of the platform allowed the rapid and secure placement of jars. Smaller, weighted platforms (3) were used for incubations under the forest canopy. Two divers could adequately set up an experiment. The following equipment was used:

a. Boat (4) and normal diving equipment; ABLJ's were essential.

b. Incubation platforms (both buoyant (1) and weighted (3) types).

c. Marker buoys (5), platform anchor (6), and ropes.

d. 450 ml 'Kilner' screw-top incubation jars with rubber injection ports set in the lids. (7).

e. Tissue cutters for lamina and stipe sections (8).

f. Diving watch and thermometer (9).

g. Pre-cut 0,5 metre squares of aluminium foil.

h. Formica data record sheet and graphite pencil (10).

i. Integrating light meters (11 - see page 39 for further details).

j. Automatic repeating syringe (12).

Experimental procedure

a. One diver secured the platform in place and positioned a marker buoy (5) near the site.

b. The incubation jars were filled with seawater at the surface to facilitate opening under pressure at depth.

c. At the site, suitable lamina discs (cut with the sharpened edge of half a tin can of diameter 6.5m, area 33.2cm^2), stipe sections (length 6cm) and entire holdfasts were cut from selected plants (A) and placed in the jars (B).

Lamina discs were allowed to fall into the bottom of the inverted jars and were thus incubated in a horizontal position (13); stipe sections were

incubated in a vertical position by pushing one end of the section onto a pin held vertically in a perspex frame (14), and holdfasts were similarly held vertically.

d. When all the tissue samples were ready for injection of isotope, the dark control jars were wrapped in foil leaving the injection ports exposed and approximately 1 ml of a 10 μCi/ml saline solution of sodium bicarbonate Cl^4 was injected (C) into all the jars from the automatic repeating syringe. The syringe operated by drawing the labelled medium through a one-way valve (15) from a reservoir syringe (16) into the delivery syringe. This system delivered volumes between 0.9 and 1.0 ml and determination of the specific activities of the inorganic carbon in the water samples taken at the end of the experiment corrected for this variation.

e. The jars were agitated by hand and then clamped into position on top of the platforms (17). The time of start of the experiment and the ambient temperature were recorded and the integrating light meters, whose readings had been noted before the commencement of the dive, were also clamped into position and switched on (18).

f. The inverted jars (with glass bottoms uppermost) were left in position for about 4 hours, usually over the midday period to avoid diurnal variations.

g. At the end of the incubation, divers took down more pre-cut pieces of foil and wrapped the light jars to stop photosynthesis. The time and temperature were recorded and the jars and light meters (which were switched off as soon as the light jars were wrapped) were recovered. When much field work was done over a short period of time in one place the buoyant platforms were often left in position for the next experiment, although those shallower than .5m were always removed due to their susceptibility to wave action.

h. The foil-wrapped jars were transported back to the shore and the foil removed in a shaded place (usually the back of a van). Water samples were taken from each jar in 28ml screw capped bottles and, in some cases, the area of tissue measured by drawing around the tissue on boards; they were

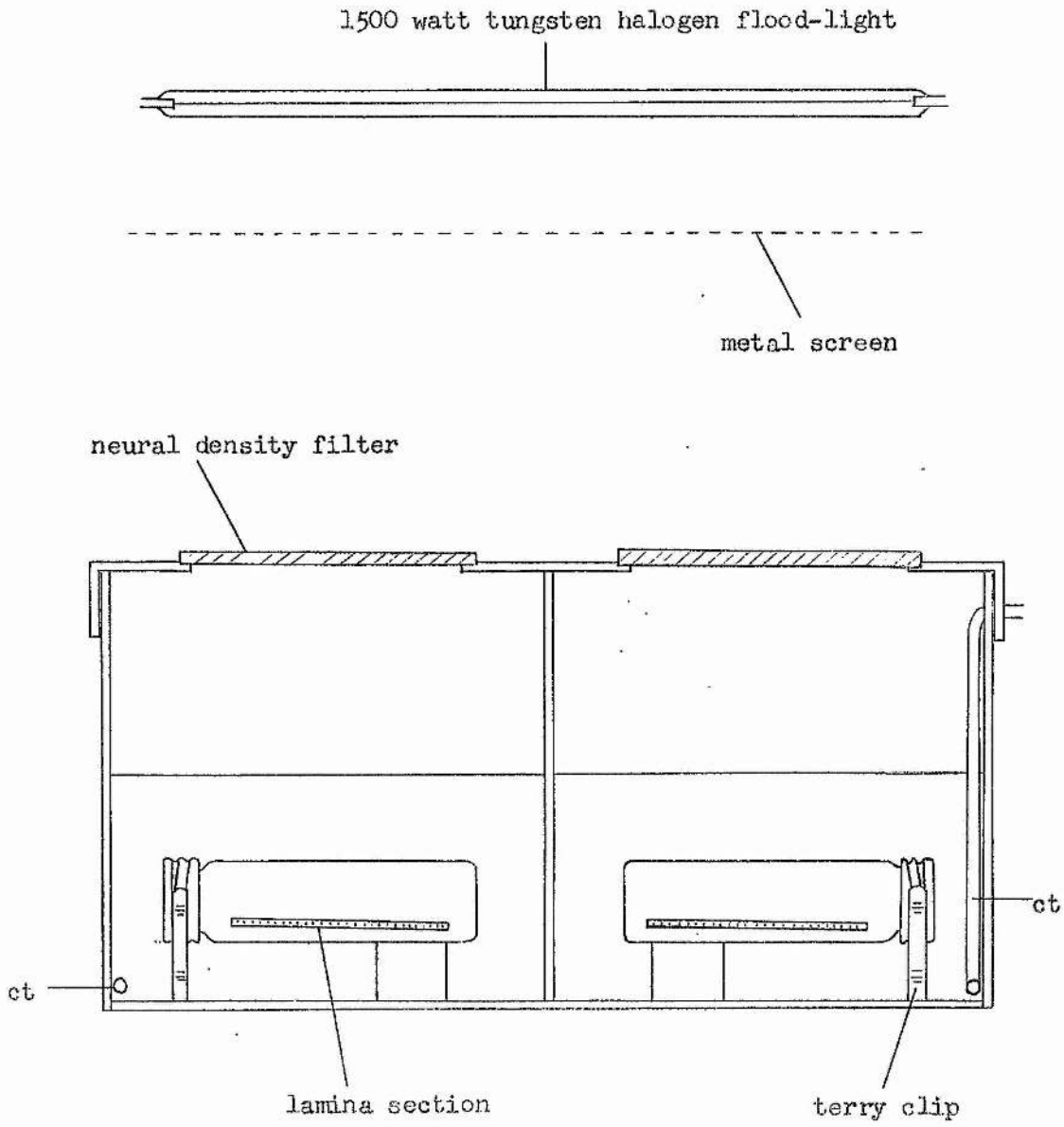


Figure 2:2 Photosynthesis apparatus used in laboratory experiments

then killed in 28ml bottles containing about 10ml 80% ethanol. The bottles were shaken to ensure adequate penetration of the alcohol and care was taken to include all adherent mucilage. All the water and sample bottles were kept in darkness. This was particularly important for the water samples in which phytoplankton photosynthesis could have removed some of the C^{14} bicarbonate, leading to erroneous specific radioactivities. All these operations were carried out over wide spillage trays and all wet and dry waste was brought back to the laboratory for disposal.

3. Photosynthesis experiments in the laboratory

These experiments were carried out in a Gallenkamp metabolic shaker bath with a light supply from one 1500 watt tungsten-halogen flood-light. A glass-bottomed cooler tank with tap water flowing through it protected the samples from the heat produced by the lamp. A neutral-density perforated metal screen was used to reduce the bright light to a reasonable intensity at the level of the shaker bath.

In the experiments on photosynthesis at various light intensities the apparatus shown in Figure 2:2 was used. This consisted of a box (26cmX22cmX13cm) made of completely opaque black perspex which was divided into four chambers. Each of these contained two Terry clips screwed into the bottom of the box so that two 28ml screw-top bottles containing the tissue (7.5 cm^2 rectangle of lamina) plus 1 µCi of sodium bicarbonate-C^{14} in the sea-water medium, could be held in a horizontal position as indicated.

The box was filled with tap water to just above the incubation bottles and a copper tube (ct) travelling around the inside of the box carried coolant medium from separate cooling unit. Experiments were carried out at 9-10°C. The hole in the lid above each chamber had a shelf recessed into its edge and one of a series of Kodax Wratten neutral density filters (2.0, 1.0, 0.5) could be positioned on this shelf so only that fraction of full light intensity transmitted by the filter was admitted to the incubation chamber. Subsequent light intensity measurements (see page 42) showed these chambers

to be light tight. The one chamber with no filter received 4.98 cal/cm^2/hr at the level of the bottles, whilst bottles beneath filter 0.5 received 33% or 1.66 cal/cm^2/hr. Bottles beneath filter 1.1 received 10% or 0.498 cal/cm^2/hr and bottles beneath filter 2.0 received 1%, 0.05 cal/cm^2/hr. The incubations were carried out using a slow shaking speed over a 1.5 inch stroke length.

pH

An analysis was made of the pH of the incubation medium at the start and at the end of one of these experiments and the results are shown in Table 2:1 .

Table 2:1 pH during photosynthesis experiments

Light intensity	Initial pH	Final pH
100%	7.59	6.75
	7.62	6.90
1%	7.64	6.32
	7.61	6.56

The drop in pH seen above is possibly due to temperature equilibration of the medium (Jackson, 1971), and since there was no significant difference between full light and 1% light treatments no buffer was added to the medium.

In a closed system such as was used in these experiments there is a danger that the carbon supply may become limiting under high light irradiances.

The total amount of available inorganic carbon in the medium is 728µg, assuming an inorganic carbon content of 26 µgC/ml and a total volume of 28 ml. In 'in situ' experiments, using 450 ml bottles, total carbon was 11,700µg.

The highest rate of gross photosynthesis recorded in these experiments was 6.4 µgC/cm^{2}lamina/h at 4.98 cal/cm^2/h. The tissue had a surface area of 7.5 cm^2 and the longest time course was 4 hours. Therefore the tissue would deplete the medium of 6.4 x 7.5 x 4 = 192 µg carbon in

this case. Assuming the highest rate of gross photosynthesis recorded in the 'in situ' experiments, i.e. 17.3 $\mu gC/cm^2/h$, the carbon requirement in this case would be 17.3 x 7.5 x 4 = 519 µg carbon in an available pool of 26x450=11,700µg.

In neither case does it seem likely that the carbon supply would limit the rate of photosynthesis even at the highest irradiance used. Thus the medium was not enriched with bicarbonate.

Levels of radioactivity.

The levels of radioactivity applied to the algal tissue were from 0.04 to 0.2 µCi/ml for periods of from three to four hours. These levels are low compared to those used by Norris et al. (1955) and Holm-Hansen et al. (1958) have shown that radioactive carbon has no direct damaging effect on biological systems at levels as high as 20% C14 to C12.

4. Heterotrophy experiment

Sections of lamina, stipe and holdfast tissue were incubated in darkness at 9°C in 5 ml of seawater containing 2 µCi of D-glucose-U-C14. The tissue was washed thoroughly in two washings (a total of 3 hours) as much of the carbohydrate 'apparently' absorbed by the alga is readily leached out again (Drew, 1969). Further details are given in Chapter 7 .

5. Respirometry

Rates of respiration of various tissues were measured at constant temperature and volume in darkness using standard manometric techniques. The 'direct' method of Warburg (see Umbreit et al. 1957) was used.

About 15 discs of tissue, diameter 7mm and thickness 2 mm, were cut from the lamina, stipe, and holdfast and submerged in 3.0ml of fresh seawater in the main chamber of Warburg flasks. Carbon dioxide evolved was absorbed by 0.2ml 10% KOH in the centre well. A roll of filter paper was placed in the centre well to aid absorption. The flasks were wrapped in aluminium foil and equilibrated at the required temperature for 30 minutes. Initial values of oxygen uptake were not used due to the complication of iodide oxidation at cut surfaces (Shaw, 1960). Results were expressed as

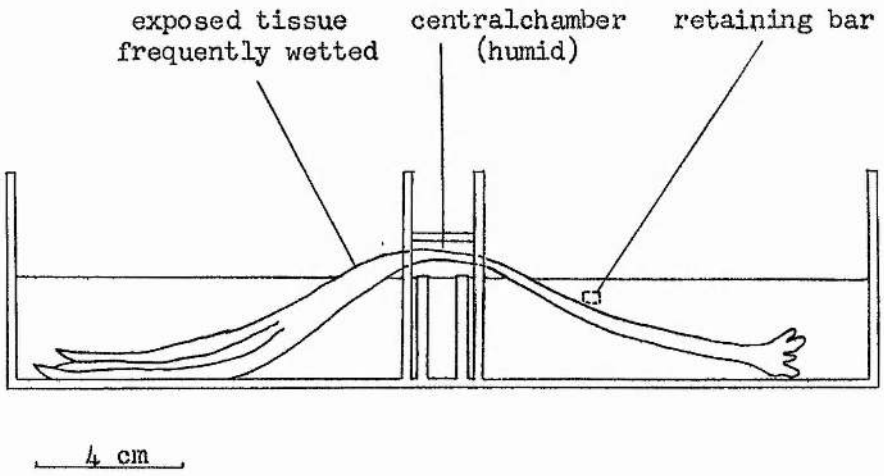


Figure 2:3 Small translocation apparatus.

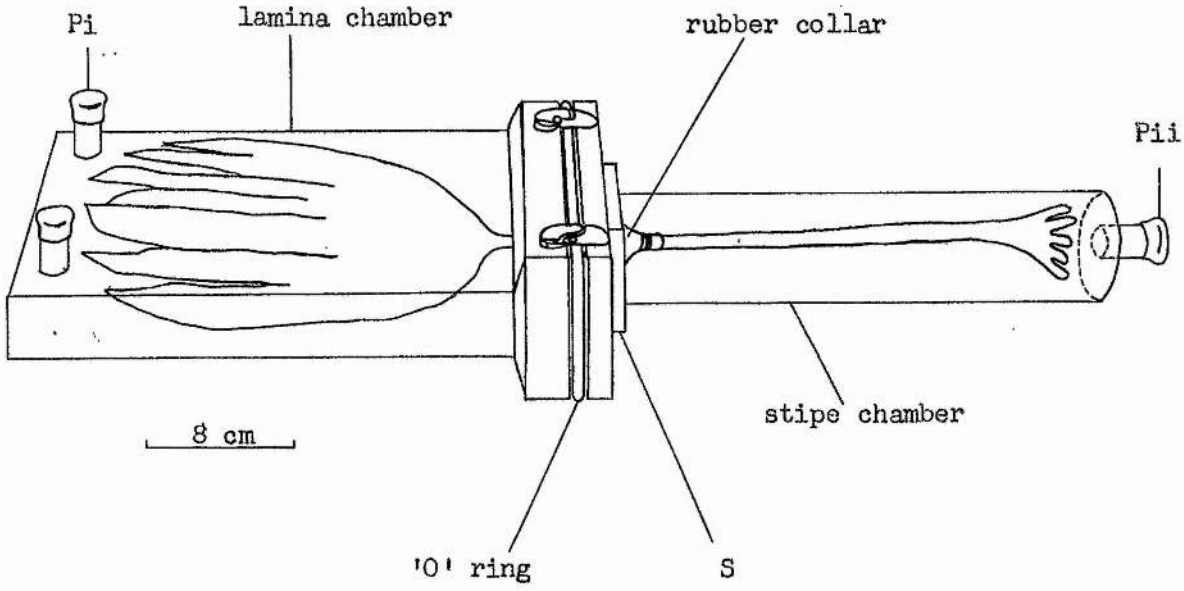


Figure 2:4 Large translocation apparatus

Q_{O_2} values in µl O_2 consumed/mg dry weight/hour, and converted to µg carbon lost/mg dry weight/hour assuming a respiratory quotient of 0.92 and that mannitol was the respiratory substrate.

6. Translocation experiments

Two types of apparatus were used to examine translocation of previously assimilated carbon 14 bicarbonate with intact plants.

a) Small open system

A clear perspex box (26cm x 13.5cm x 5.5cm), illustrated in Figure 2:3 was used to investigate translocation of radioactive assimilates from the old lamina to the new lamina and also from the stipe to the new lamina of L. hyperborea. Small plants were used, usually 10 to 15 cm long, collected from Fife Ness and either used immediately or subjected to dark starvation treatment.

There was some danger of desiccation of tissue in this apparatus and efforts were made to cut this down using a central partition to maintain high humidity around that part of the lamina draped from one chamber to the other. The tissue exposed in the incubation chamber (not more than 1-2 cm) was moistened at frequent intervals with seawater from a syringe. Aerial diffusion of exchanged C14 and consequent contamination of the unlabelled chamber, was found to be high when a lid was used on the box so subsequent experiments were carried out without the lid. Further details are given for each experiment in the results section. In most cases, incubation was carried out for 2-4 hours at 10°C and a light intensity of 4.98 cal/cm^2/h.

b) Large, closed system

The apparatus shown in Figure 2:4 was designed to seal off carbon 14 labelled water medium from the unlabelled water in translocation studies with plants of L. hyperborea up to 60cm long. This system had the advantage over the previously described apparatus that the desiccation intolerant tissues of the alga were completely immersed. The apparatus was constructed of

clear perspex. When setting up an experiment with this apparatus the stipe, with most of the haptera removed, was first fed into a rubber collar cut from the region of a balloon where the narrow inflation tube starts to widen. The collar was held tightly onto the stipe with a rubber band wound onto a match. The stipe was then fed into the long tube (stipe chamber). The wide flange of the collar was then pressed out against the shoulder (S) at the end of the stipe chamber. An incomplete perspex ring was fitted between the collar and the stipe and a nylon screw turned carefully into the milled end of this ring; this caused it to expand outwards and tighten the collar onto the shoulder of the stipe tube. These regions were also greased to cut down water leakage. The lamina was fed into the lamina chamber and the two chambers clamped down over a large, greased 'O' ring. The two chambers were then very carefully filled with seawater so that no appreciable head of water pressure built up in one compared to the other. When complete this left a plant completely immersed in seawater in two chambers which were isolated from each other by a collar and, although some leakage was found, this apparatus was found to work satisfactorily.

The whole apparatus was placed onto a dexion frame in a large water bath so that the upper side of the apparatus was 10.5cm below the surface of the water. A cooling unit maintained the temperature of the water at $5^{o}C$. It was illuminated at an intensity of 3.13 cal/cm^2/hr at the level of the apparatus being supplied by a spectrally balanced battery of two 5' daylight fluorescent tubes, two 4' warm white tubes, and two 4' "Grolux" tubes, suspended 22cm above the surface of the water. See Figure 2:5 for spectrum produced.

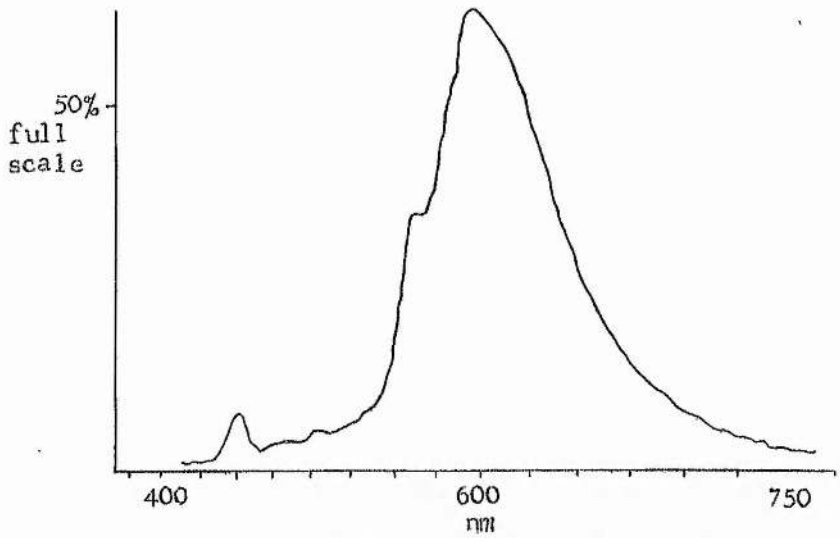


Figure 2:5 Spectrum of lights used in translocation experiments

After an initial equilibration of the plant under the lights the apparatus was removed and 100 μCi of NaH CO_3-Cl_4 in 1ml. of seawater was injected into either the lamina chamber (volume of 2 litre) or the stipe chamber (volume of 1 litre) through the ports Pi Pii, After the injection a bubble of air left in the incubation chamber was used to mix the isotope throughout the chamber taking care not to build up pressure at the lamina/ stipe collar. Lateral oscillation of the lamina chamber was found to mix dichromate dye adequately when this was injected in the same way in a test experiment. The similar amounts of Cl_4 fixed at various distances along the lamina or stipe in the fixation chamber was a further indication of good mixing (see Figure 9:2). The apparatus was occasionally shaken during an experiment which lasted up to 51 hours.

7. Cultures

Cultures of small sporophytes were maintained in tanks under lights in a cold room. The cultures of sporophytes of L. hyperborea were kindly sent by Dr. J.M. Kain (Mrs. Jones) of the Marine Biological Station (University of Liverpool), Port Erin, Isle of Man and were grown on 2 inch glass slides from spore suspensions using the methods of Kain (1964). The slides were air-freighted (journey of 3-4 hours) in plastic slide containers which were filled with seawater, sealed around the lid, and laid horizontally in a polystyrene box. Two freezing bags were placed around the slide containers to maintain low temperature.

After collection, the slides were carefully removed from the containers, transferred to small tanks and covered with about 600ml of medium. The medium used was basically that of Kain & Fogg (1960). Fresh, filtered seawater from the middle of St. Andrews Bay was used and enriched with 1.0 mM KNO_3, 0.1 mM K_2HPO_4, 0.005 mM $FeCl_3$, and the '12 V' vitamin mixture of Kain & Fogg (loc cit), excluding ascorbic acid, and a soil extract.

Four litres of medium were made up at a time and the KNO_3, $FeCl_3$, soil extract (200ml), and vitamin stock (2ml) were mixed and autoclaved first;

the K_2HPO_4 was added after cooling because it precipitates in hot solution. This medium was stored cold and used to change that of the cultures every two weeks.

The culture tanks were enclosed within black polythene sheeting and in the high light culture - 1.78 cal/cm^2/hr - three layers of muslin were placed across the top of the tank which was maintained at 10°C in a cold bath; illumination was provided by 1 Osram 'daylight' fluorescent tube suspended 17 cm above the tank. The low light cultures were maintained at 10°C under a 'daylight' tube at 20 cm above the tank and the light intensity was reduced to 0.22 cal/cm^2/hr at the level of the slides by 8 layers of muslin. Air was bubbled gently into the medium of both treatments from a pipette kept some distance from the slides. Plate I shows high and low light cultures plants photographed 10 days after collection.

Analytical procedures

1. Biometric data and computer analysis.

The samples of plants from the quadrat collections were analysed in the following way:

a) all the epiphytic and epizoic growth on the stipes and holdfasts were carefully scraped off with a knife.

b) the plants were divided into lamina, stipe, and holdfast, the lamina was subdivided into old and new lamina, if present, and the two were treated separately.

c) the stipe was taken as that portion between the point of expansion of the lamina and the upper limit of the haptera. The length of stipe thus recorded was less than that of Kain (1963), who included the haptera-cleared stump in her measurements, but it is the same as that used by John (1968) and Whittick (1969).

d) the area of the lamina was measured by spreading the tissue flat on a 1 m^2 board marked off in 5 cm squares, each of area 25 cm^2, and counting the number of squares and parts thereof covered by the lamina.

e) age determinations were made by making a 1mm transverse section of the

stipe just above the latest haptera to include the most recent growth ring (Kain, 1963). The annual rings formed by the activity of the secondary meristem were then counted to give an estimate of the age. Kain (loc cit) criticises the use of transverse sections (used also by Black et al., 1959) as the boundary of primary and secondary tissue cannot be distinguished unless followed to the base using a longitudinal section but as Kain (1971) points out the great problem of 'interference' lines makes all ageing methods suspect so the results obtained must be only considered within the limitations of the method used.

f) the fresh weight of the tissue was measured and roughly corresponded to the 'wet' weight of Westlake (1963) i.e. the weight of tissue with some of the adhering water shaken off during transport and handling.

g) the tissues were then cut into smaller sections, wrapped in aluminium foil and dried to constant weight in an air-circulating oven at 80°C. Weighings were carried out on a Mettler single-pan balance of 700g capacity.

h) Analysis of data

All the data for the following parameters was punched onto cards with each plant and quadrat receiving a code number:-

Parameters analysed

Age; fresh and dry weight of holdfast, stipe, and lamina; stipe length; area of new and old lamina; specific lamina area on a fresh and dry weight basis for old and new lamina.

The data was analysed using the IBM distributed subroutine MISR from a scientific subroutine package. The programme was run on an IBM 360/44 computer, selecting the maximum, minimum, mean, standard deviation, and total for each parameter in the following ways:

(i) The data was considered in two blocks,

a. 'Shallow' data - all quadrats from 3.1 m.

b. 'Deep' data - all quadrats from 9.1 m.

(ii) Statistics for all parameters within each quadrat irrespective of age.

This gave biomass data for comparing the populations at 3.1m and 9.1m and also for considering seasonal increments in biomass.

(iii) Statistics for parameters within each age group of each quadrat. Data was insufficient to consider growth of each age group over the season but data for older age groups was used.

(iv) Statistics for parameters in each age group irrespective of quadrat was found. This ignored time of cropping and allowed consideration of the data over the life span of the plant. This method of treating data would be suspect where large seasonal variations occurred in the data considered such as with the lamina, but it was considered valid for holdfast and stipe tissue which do not vary greatly over the season. (Black, 1950a).

2. Bomb calorimetry

Sub-samples of the oven-dried lamina, stipe, and holdfast tissues were ground to a fine powder in a mortar. 1.0g of this material was accurately weighed in a previously tared nickel crucible which was placed in the barrel of a Gallenkamp CB-370 Ballistic Bomb Calorimeter. The sample was ignited at 25 atmospheres oxygen pressure and the maximum deflection noted on a galvanometer connected to a thermocouple in the 'bomb' barrel. This deflection was corrected for heat loss produced by the firing thread, and was converted to kcal/g by comparison with the deflections caused by thermochemical grade benzoic acid (6.32 kcal/g). Assuming that the temperature did not exceed 550°C at which temperature $CaCO_3$ may be decomposed, (previous experiments by W.A.A.Robertson, pers. comm., indicated that this did not occur) the residue left in the crucible after ignition represents the ash contents of the tissue. Subtraction of this weight from the 1.0g dry weight used in the ignition gave the amount of ash-free organic dry weight in the material. This figure was used to convert dry weights obtained in biometric studies to organic weight.

3. Extraction and hydroysis of algal material

It was necessary to fully extract soluble material from the bulky

tissues of Laminaria in samples from 'in situ' and laboratory experiments for radioactive assay and also in samples for gas-liquid chromatography. Direct counting of such thick tissue on planchets was not satisfactory. At the end of experiments tissues, with any adhering mucilage, were rapidly killed in 80% ethanol contained in 28 ml screw capped bottles and left overnight. Complete extraction was achieved with a further three changes of hot 80% ethanol. In samples tested, the radioactivity in the last change of ethanol was only 5% of the total radioactivity extracted in ethanol. All the ethanol extracts from each sample were bulked and made to known volume and either the entire extract or 25ml aliquots were kept in 28 ml screw capped bottles. This was termed the 'ethanol soluble fraction'.

Corrections for weight of material in soluble fraction

In the case of gas-liquid chromatography of carbohydrates in the ethanol soluble fraction it was necessary to calculate the weight of alcohol soluble substances (Holligan & Drew, 1971). Thus the dry weight of 10ml aliquots of extracts of lamina, stipe, and holdfast were found by drying down the aliquots slowly in crucibles. Once the dry weight was recorded the salt content in each extract was found photometrically by taking up the salt in 2ml of distilled water and, after appropriate dilution, reading the chloride reaction with mercuric thiocyanate in the presence of ferric ions (BDH chloride colour reagent) at 440nm. in a Unicam SP 600 Spectrophotometer. The milliequivalents of chloride present was calculated from standard solutions. The amount of mannitol in each extract was calculated from the GLC scans and the total weights of mannitol, salt, and other alcohol soluble substances, if any, are set out in Table 2:2.

Table 2:2

Dry Weight of materials in ethanol soluble extracts (mg)

Tissue	Extr. dry wt.	total dry wt. in extract	Mannitol	Salt	Total wt. M+S
Lamina	290.4	61.5	12	19.8	31.8
Stipe	218.5	* 93.6	76.5	31.8	108.3
Holdfast	1069.4	182.5	125	55.8	180.8

*possible losses during evaporation.

As can be seen the majority of weight in the alcohol soluble extracts of stipe and holdfast materials was due to mannitol and salt so that a correction was made to the extracted dry weight of these tissues by adding on the weight of mannitol found in the ethanol extract.

However, in the case of the lamina material, it is apparent that some 50% of the dry weight in the ethanol extract cannot be accounted for by mannitol and salt and this weight must represent other organic materials (e.g. pigments) which are soluble in 80% ethanol. A correction factor of x0.144 of the extracted dry weight of tissue was used to account for these other materials.

Acid hydrolysis

The residue remaining after ethanol extraction was often a large percentage of the total dry weight and the data in Table 2:2 suggests that it may be from 60% to 90% of the total weight. This material was often bulky, especially the holdfasts (see above) and it was necessary to hydrolyse it to determine the remaining radioactivity in it and also to examine glucose residues from laminarin.

The ethanol extracted material was dried to constant weight in aluminium cups in an air-circulating oven at 80°C. In some cases where the area of tissue was not known before extraction the area of the ethanol extracted tissue was measured and a factor applied for the shrinkage of tissue in ethanol. The alcohol extracted tissue was then hydrolysed in 5-10 ml of 1N H_2SO_4 at 100°C for 3 to 4 hours. The hydrolysis was carried out in long test tubes with a foil cap over the end to condense any water vapour from the hydrolysis.

A control experiment was carried out in which a solution of D-glucuronic acid was hydrolysed under exactly the same conditions as the experimental tissue. Glucuronic acid was tested since it is an epimer of L-guluronic acid which is a constituent, with D-mannuronic acid, of alginic acid (Florkin & Stotz, 1963; CIBA Review, 1969). A standard solution of

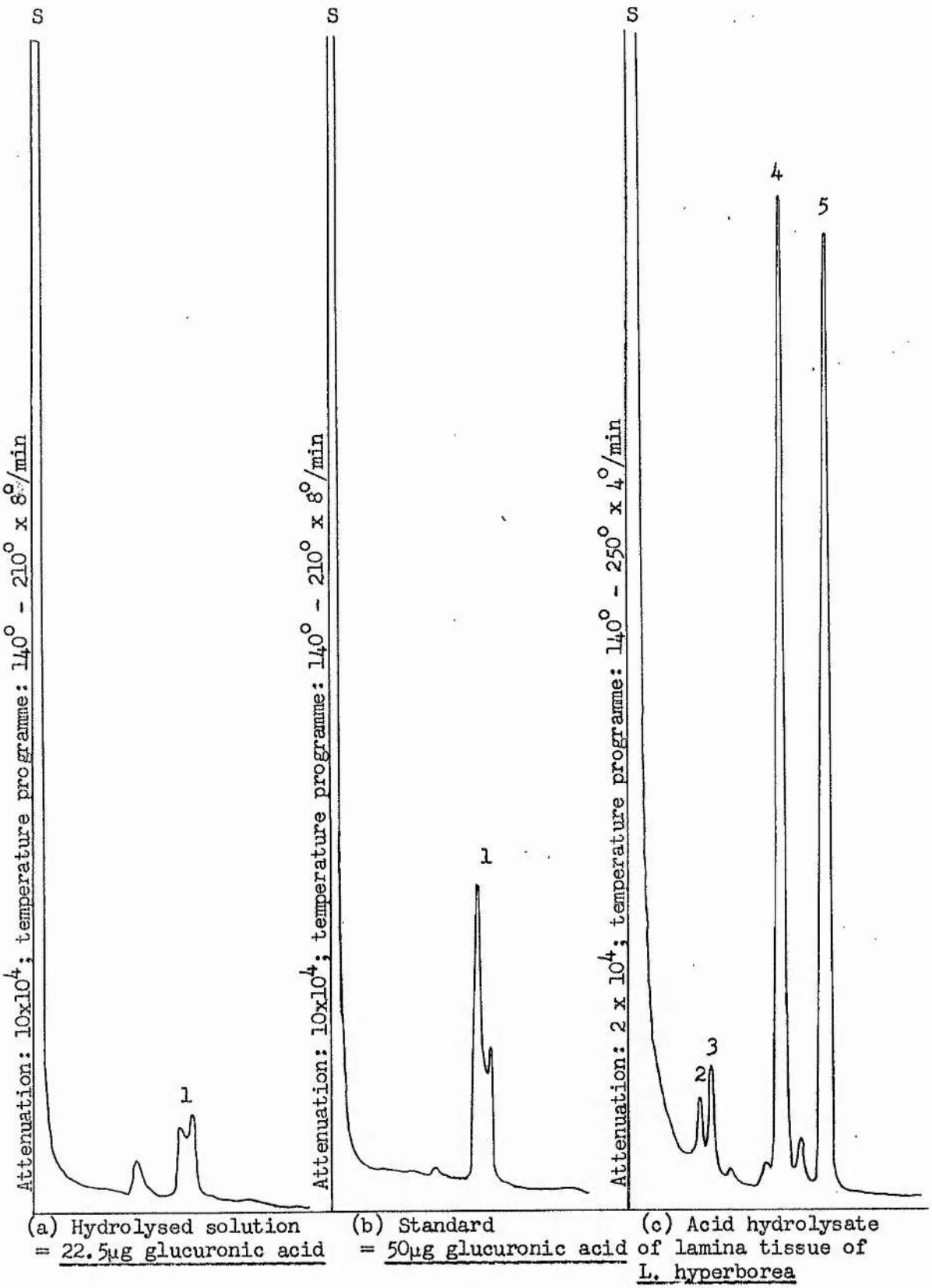


Figure 2:6 GLC traces using a 5' glass column packed with 2% SE52 on Diatomite 'C' (60-72 mesh) and nitrogen carrier gas (60 ml/min. and 5 p.s.i.)

50 mg glucuronic acid in 20 ml water was made up and a 5 ml aliquot (12.5 mg) was hydrolysed with 5 ml 1N H_2SO_4 at 100°C for 3 hours. Four ml of the neutralised hydrolysate, which should theoretically contain 5 mg glucuronic acid was dried down and sylylated with 1 ml of the sylylating reagents (see gas-liquid chromatography). 10µl of this reaction mixture was injected onto the GLC column and the scan with a standard are shown in Figure 2:6 . Both peaks should represent 50 µg of glucuronic acid. However, the hydrolysed solution contains only 22.5 µg of glucuronic acid. It would appear that the acid hydrolysis is breaking down uronic acid residues to possibly furfurals, carbon dioxide, and water (see Percival in Florkin & Stotz, 1963). That a considerable breakdown of ethanol insoluble compounds occurs during this hydrolysis is also indicated by the finding that the dry weight of the residue after hydrolysis was usually only 50% of the extracted dry weight. It would appear that this treatment degrades large amounts of alginic acid.

The effect of this treatment on other compounds was not tested but the large amounts of glucose and some fucose found in the acid hydrolysate (see GLC scan, Figure 2:6) indicate that this treatment breaks down laminarin and fucoidin. Cameron et al. (1948) estimated that N sulphuric acid hydrolysis at 100°C of laminarin was complete in 4.5 hours. Yamaguchi et al. (1966) and Bidwell (1967) used similar treatments to break down polysaccharides in brown algae; Yamaguchi et al (loc cit) found considerable amounts of fucose, xylose and glucose in the acid hydrolysate of their 'laminaran fraction'. It is assumed that the acid hydrolysis used here broke down all the laminarin present into its constituent monomers, D-glucose and D-mannitol (Florkin & Stotz, loc cit). After hydrolysis was completed the hydrolysate was carefully decanted into a 28ml screw capped bottle and the residue washed several times with distilled water. All solutions were bulked and made to known volume, (usually 10 ml). The residue left was soaked in excess distilled water to remove any traces of acid and stored at 2°C until counted.

4. Radioactive assay

(i) Gas flow counting

A. Sample preparation.

The samples of ethanol soluble, acid hydrolysate, and residues from the Cl4 experiments were prepared for counting in the following way:

a) Ethanol soluble fraction

0.1ml aliquots of ethanol extracts were dried down on planchets (2.5cm diameter) together with a drop of glacial acetic acid to remove any traces of sodium bicarbonate-Cl4 left in the extract. The planchets were first ringed around the outer edge with a wax pencil on a warm hot-plate so that the aliquots did not 'creep' up the edge of the planchet. The aliquots retained in the centre of the planchets were slowly dried down on a slide warming plate at $40^{\circ}C$.

b) Acid hydrolysates

A 2ml aliquot of the acid hydrolysate was neutralised in a centrifuge tube with excess barium carbonate. The end-point was checked with litmus paper and the neutralised hydrolysate was centrifuged. A 0.2ml aliquot of the clear supernatant was dried down on a planchet; no wax ring was necessary for these samples.

c) Residue

The final soaking water was decanted off and in early experiments the entire residue was pulverised and counted after spreading on a number of planchets. Subsequently an aliquot method was used in which the residue was completely macerated in the hydrolysis tube and 2 aliquots of this damp residue taken spread thinly on planchets and dried down. The aliquots and remaining residue dry weights were estimated. The aliquots often curled up so they were transferred to other planchets with pieces of double sided sticky tape and pressed firmly down so no tissue stuck up. (flat geometry was essential for the counting system used).

Water Samples

The inorganic carbon in the seawater samples was precipitated as

$BaCO_3$ by adding 1ml of saturated $Ba(OH)_2$ to 1ml of the water sample in a centrifuge tube. The precipitate obtained after centrifugation was washed twice with hot distilled water to remove traces of barium hydroxide. The precipitate was resuspended in 1ml of distilled water and 0.2ml was plated onto a planchet and dried.

The inorganic carbon content of seawater was found to be 26 µg carbon/ml (unpublished data of Drew), using a volumetric determination of the Co_2 released from seawater with acid.

B. Counting equipment

a) Gas-flow proportional counting system

All the planchets prepared as described were counted on a Nuclear Chicago automated gas-flow proportional counter with a 50 sample capacity. The gas used was 90% argon: 10% methane and the equipment was characterised by a short dead-time (6 µ sec), low background (10-17 cpm), and an efficiency of about 20% for C^{14} when thin end windows were used.

Planchets were each counted for three 10 minute periods. The background cosmic radiation was estimated with one clean planchet in each counting series.

Statistical estimate of mean background

In translocation experiments counts were often one or two above background and a statistical test of the significance of such small count rates was necessary. Thus a total of 138 backgrounds, each a mean of three as above, were analysed for mean, standard error of mean, and the 95% confidence limit, and this data is set out in Table 2:3 .

Table 2:3

Statistics for counting background

Total number of samples	n	= 138
Mean	x	= 13.4
Standard errors of mean	SEM	= 0.128
95% confidence interval	CI	= 0.25

(using value of t for 120 degrees of freedom)

Thus it can be said that values greater than 1 or 2 counts above background are significant.

Corrections

Counts were first corrected for background and, in cases of very high counts, for dead time using the formula:-

$$R' = \frac{R}{1 - Rt}$$

where R' = number of counts that would be recorded if there were no dead time.

R = observed counting rate.

t = dead time.

Self absorption losses were not necessary for low density ethanol extracts and acid hydrolysates but were needed for the denser barium carbonate precipitates and the tissue residues. The counts obtained were corrected to 'infinite thinness' using a correction curve drawn from the data of Drew (pers. comm.), using a nomogram for Beta particle absorption in the Radiochemical Manual, 1966. Some examples of the correction factors for various densities of material are given in Table 2:4

Table 2:4

Self absorption correction data.

Density (mg/cm^2)	Correction factor
2.5	1.4
5.0	1.8
10.0	2.7
20.0	5.0
30.0	7.4
40.0	10.0
50.0	12.5

(ii) Liquid scintillation counting

In view of the low counts sometimes experienced, liquid scintillation counting, with its greater inherent efficiency, was used. The samples of algal tissue were prepared for counting in the following way.

The tissue was counted as a suspension in glass vials. Difficulties were experienced in macerating the tough, mucilaginous algal tissue into a homogenous suspension. The usual solubilisers such as NE 520 were inadequate in this case so tissues were hydrolised in 0.7 to 1.4 ml of 1 N H_2SO_4 for 3 hours at 100°C and macerated with a high speed mixer.

The scintillation solution was made up of 0.5 ml to 2.0 ml of the sample, plus 1 ml NE 520 (solubiliser), plus 5-10 ml of NE 233 (toluene based scintillator). The sample suspension settled onto the bottom of the vials and vials were kept in darkness prior to counting. A background consisted of only NE 233 and NE 520 and a seawater sample was counted with 1 ml water plus 1 ml NE 520 and 10 ml NE 233.

Counting equipment

Counts were made for 10 minute intervals in each of the following gates and settings, on a model C3320 Tricarb Liquid Scintillation Spectrometer (Packard Instrument Co.).

			Discriminator Intervals	Gain settings %
Gate	A - B	Red	50 - 1000	7.85
Gate	C - D	Green	50 - 1000	7.85
Gate	E - F	Blue	1000 -	6.0

Quench correction

Many of the samples gave large reductions in the external standard count rate compared to the blank value so a quench correction curve was made using varying amounts of the algal macerate as the quenching agent and equal amounts of C^{14} bicarbonate and Scintillator and varying amounts of a non-quenching agent, toluene was used, so that the final volume was the same in each vial. The volumes and counts in the three gates for the external standard are set out in Table 2:5 .

Table 2:5

Volume sample	Volume non quencher	^{14}C NE233	cpl0m x 10^{-3} Automatic External Std. Red	Green	Blue
0.0	1.0	10µl 10ml	771	773	316
0.1	0.9	" "	762	764	324
0.2	0.8	" "	759	761	322
0.3	0.7	" "	752	754	322
0.4	0.6	" "	756	758	324
0.5	0.5	" "	767	769	326
0.6	0.4	" "	774	776	328
0.7	0.3	" "	755	757	328
0.8	0.2	" "	782	784	317
0.9	0.1	" "	762	763	313
1.0	0.0	" "	760	762	325

As can be seen increasing amounts of the quenching agent, the sample, has very little effect on the counts given by the external standard. This is probably due to the intense colour quenching from each sample. Thus it has been assumed that quenching is the same for each sample counted and amounts of sample up to 2.0 ml per vial do not quench significantly more than amounts of 0.5 ml sample per vial. This means that the efficiency of counting does not vary with the different amounts of sample so the counts obtained were corrected back to 100% using the quoted counting efficiency of the machine for C14; i.e. 90% (Packard Instrument Co. Ltd.).

5. Autoradiography

X-ray film autoradiography was used to detect radioactive compounds on chromatograms and also to detect radioactivity in tissue from translocation experiments. No protection was necessary against chemography in the case of paper chromatograms but protection was essential in translocation experiments.

The chromatograms or whole plant sample was placed between two 6" x 15" (15.2 x 38.1cm) Kodak 'Crystallex' (estar base) films all operations being carried out in a photographic dark room under a brown safelight, Wratten 6B. A 'sandwich' was made by pressing the films between glass plates, taping round the edges and putting the glass plates between formica boards. The

complete 'sandwich' was then wrapped in black polythene and kept in the cold room for exposures ranging from 1-3½ months.

The film was developed with Kodak D-19 developer, a high contrast quick acting developer, for 5-10 minutes at 20°C with agitation every few minutes until fogging was seen. The film was then rinsed for 2 minutes in running tap water and transferred to sodium hypochlorite for a further 10 minutes with constant agitation. The film was then washed and hung up to dry in the darkroom.

6. Paper chromatography

Descending paper partition chromatography was used for the separation of carbohydrates in ethanol soluble extracts. Whatman No 1 and 3MM papers were used and chromatograms were run in Shandon all-glass two dimensional tanks at room temperature.

Ethanol extracts were first dried down in small crucibles at room temperature and applied as small spots with Pasteur pipettes or with 50 µl Drummond 'Microcaps'. Spots were confined to 1 cm diameter with a cold air stream from a Philips hair drier. In some cases an automatic delivery system was used so that the small aliquots of sample could be held in a reservoir and spots applied at the operator's convenience.

Solvent systems

Two solvent systems were used.

Ethyl methyl ketone, acetic acid, saturated boric acid (9:1:1)

This solvent is that of Rees and Reynolds (1958) and separates sugar alcohols from their corresponding sugars. The compounds run as borate complexes.

Tertiary butanol, picric acid, water

2.2 gm of wet picric acid was dissolved in 100 ml of 80% t-butanol (Hanes & Isherwood, 1949). This solvent separates mixtures into organic phosphates, carbohydrates, amino and organic acids, with the solvent front still on the paper.

Identification of compounds

Authentic marker spots were run simultaneously with the unknown

compounds and similar mobilities to the marker in a given solvent system were taken as an indication of chemical identity.

Detection of radioactivity on chromatograms

Two methods were used to detect radioactivity on paper chromatograms: strip scanners and X ray film autoradiography (see section 5).

Strip scanners

Strips of chromatography paper 4 cm wide were passed through either a Nuclear Chicago Actigraph 4π scanner or a Tracerlab 4π scanner.

7. Gas-liquid chromatography

For the routine quantitative analysis of carbohydrate content in Laminaria the technique of gas-liquid chromatography was used. A Pye Unicam Automatic Preparative Chromatograph (Series 105; model 15) was used in an analytical form, and gave accurate and sensitive estimations of carbohydrates within 30 minutes of injection of sample. (see Holligan, 1971 & Drew, 1971 for details of the capabilities of this system). The apparatus consists of a 5' x 1/4" internal diameter glass column with a glass-metal outlet to a flame ionisation detector. The detector was used in conjunction with a Leeds and Northrup 'Speedomax' pen recorder with a disc integrator.

Column packing and support material

The support phase was acid washed, siliconised Diatomite 'C' (60-72 mesh) and the liquid stationary phase was 2% SE 52 (phenyl silicone gum). These were prepared as described in Holligan & Drew, (loc cit). The columns were packed by application of a moderate vacuum at the outlet.

Preparation of volatile derivatives.

The volatile trimethylsilyl (TMS) derivatives of sugar alcohols and sugars were prepared by redissolving the dried ethanol extracts (2ml) in 0.85 ml pyridine, and adding, within 5 minutes, 0.1 ml Hexamethyldisilazane (HMDS) and 0.05 ml trimethylchlorosilane (TMCS) to give a reaction volume of 1.0 ml (Sweeley et al. 1963). After shaking vigorously the reaction mixture was allowed to stand overnight at room temperature before analysis to give a constant time for reaction.

Injection of samples and column programme

10µl aliquots of the silylated derivatives were injected rapidly onto the column at 140°C. A temperature programme from 140° to 250°C and a temperature rise of 4 or 8°C/minute was used. The carrier gas was Oxygen free nitrogen (70 p.s.i. head) with 5 p.s.i. at the column and a flow rate of 60 ml/minute. Hydrogen (12 p.s.i.; 30 ml/min.) and air (60 p.s.i. 500 ml/min) were supplied to the flame ionisation detector.

Identification and quantitative estimation of peaks

The retention time from the peak apex to the solvent front was measured and similar retention times to authentic standards were considered as evidence of chemical identity. In the main the sugar alcohol mannitol was investigated although in Chapter 7 the sugars in hydrolysates were also investigated and here co-chromatography of an unknown with authentic standard was used to identify the unknown compound.

The peak areas were determined from the response of the disc integrator of the recorder, correcting for base line error. The linear response of the flame ionisation detector and the disc integrator for calibration curves with TMS derivatives of standard sugars shown by Holligan & Drew (loc cit) indicate the accuracy of this technique. However, in the case of glucose estimations in Chapter 7 the base line error was large compared to peak height so the more reliable measure of peak height x width of peak at ½ peak height was used (Ball et al, 1967).

8. Pigment analysis

The techniques used to analyse pigments in L. hyperborea are essentially those described by Goodwin (1965).

Preparation of material

Field samples were collected from 3.1 m and 10.7 m at Fife Ness During recovery, theplants were wrapped in sacks and put in tanks of seawater in the boat to avoid any light-induced changes during transit to the shore. At the shore 6 x 6 cm sections were cut from healthy, epiphyte-free sections

of the lamina of three plants from each depth. The discs with the appropriate holdfast for ageing purposes, were placed into foil wrapped, labelled Kilner jars filled with seawater.

At the laboratory 2g fresh weight of lamina tissue was cut from the discs, the area drawn around on paper and the tissue wrapped in foil and stored in a deep freezer at -40°C until analysed (Holden p.463 in Goodwin, loc cit, recommends freeze drying tissue in this way).

Extraction of pigments

The tissue was extracted in a cold room at 5°C with a minimal amount of light to work with. The algal material was found to be difficult to extract with normal grinding techniques so the following procedure was adopted:

The material was cut up into smaller pieces with scissors in a mortar (all apparatus was chilled prior to use) and a few mg of $MgCO_3$ were added to prevent the formation of phaeophytin. 1ml of distilled water was added to assist lysis of cells (Black, 1950b). A few ml of liquid nitrogen (highly recommended for extracting pigments from mucilaginous algae) was poured over the tissue which immediately froze solid and cracked up into smaller pieces. This still-frozen tissue was macerated with a pestle after 5ml of absolute acetone was poured onto it. Maceration was carried out for 5 minutes fairly gently so that the hard pieces of alga did not fly out of the mortar. A high concentration of acetone was needed right from the start to deactivate chlorophyllase (Barrett & Jeffrey, 1964). The extract was centrifuged and the supernatant was decanted into pre-frozen 28 ml screw capped bottles which were wrapped in foil and stored at 2°C. The residue was returned to the mortar and re-extracted a further 5 times with 5 ml aliquots of 90% acetone. All the acetone extracts were bulked, made to 25ml and stored at -40°C until analysed.

Recovery of pigments

Working in subdued light the acetone extract was transferred via a dropping rod into a separating funnel and an equal volume of diethyl ether (kept in the dark to prevent peroxide formation) was added. The transfer of

pigments from the acetone to the ether layer was aided by gentle shaking for a few minutes. A few ml of distilled water were added carefully to avoid emulsification and the lower, aqueous layer was run off and re-extracted with ether. This extract was passed back into the funnel and the ether extract washed twice more to remove any traces of acetone.

The final aqueous layer was run off and discarded. The ether extract was collected in foil wrapped 'Quickfit' flasks (50ml and dried down 'in vacuo' at room temperature, taking about 3 hours for a 40ml ether extract. The dried pigments were taken up in five 5ml aliquots of diethyl ether, made to 25ml and stored under nitrogen in McArtneys in the deep freeze at minus 40°C.

Quantitative determination of pigments

Thin layer chromatography was used initially, as described by Riley & Wilson, 1965, 1967) but as losses occurred, multi-chromatic mixtures were used in spectrophotometric determinations.

Apparatus

Absorbance was measured in an automatic scanning Pye Unicam SP 1800 Ultra-Violet Spectrophotometer. This machine has a three sample holder and an automatic scan over the spectral range 390nm to 690nm was used; a didynium cell is fitted into this apparatus to compensate for the change over point at 550nm. The scan was recorded on a synchronous AR 25 Linear Recorder.

1. Chlorophylls

Absorbance was measured in both ether and acetone.

Ether extracts

A few ml of each ether extract were placed in 1cm cuvettes and from the absorption curve, total chlorophyll was determined using the formula (in Goodwin, loc cit) :

$$\text{Total chlorophyll (mg/l)} = 100.5.\ D_{600}$$

where D_{600} is the absorbance at 600nm.

Acetone extracts

A 1 ml aliquot of the ether extract was dried down 'in vacuo' in foil

wrapped Quickfit pear-shaped flasks and taken up in 5 ml of 90% acetone. The absorbance at 630nm and 665nm was read and the concentration in mg/l of chlorophyll a and chlorophyll c calculated from the following formula due to Parsons & Strickland (1963):

$$\text{Chlorophyll a} = 11.6.\ E_{665} - 0.1\,4_{630}$$

$$\text{Chlorophyll c} = 55.0.\ E_{630} - 4.64_{665}$$

The ratios for chlorophyll b were taken out of the original formula.

2. Carotenoid

The major carotenoid was fucoxanthin. Absorbance was measured after drying down a 1ml aliquot from the ether extract and taking up the dried pigment in 5 ml of CS_2. The formula used was

$$x = \frac{E.y}{\frac{1\%}{E_{1\ cm}} \times 100}$$

The $E^{1\%}_{1cm}$ value used was that quoted in Goodwin (loc cit); i.e. 2,025 at 477 nm. Where x is in gm, y in ml, and E the absorbance at λ max. i.e. 477 nm, the value for carotenoid was further checked using the formula of Strickland (1965):

$$\text{Carotenoids (MSPU/l)} = 10\ E_{480}$$

where the MSPU (or millispecific pigment unit) corresponds to the milligram.

All the values obtained from the above formulae were converted to original volume and expressed as mg pigment/g fresh weight of tissue.

9. Electron microscopy

The electron micrographs in Plates II to V were prepared by Dr. L.V. Evans, Department of Plant Sciences, University of Leeds from cultured and field material sent to him. Fixation staining, and embedding were as described in Evans (1966) except that 5% glutaraldehyde without buffer or sucrose was used, and a propylene oxide / Epon 812 mixture was used for embedding.

10. Light measurements

Light intensity (irradiance) was measured both under field conditions, during 'in situ' experiments, and in laboratory experiments using various fluorescent lamps.

Nomenclature and conversion factors

The nomenclature used is that of Withrow & Withrow (1956). Light intensity, known as irradiance (radiometric term) or illuminance (photometric term), is defined as power per unit area and is measured, in this thesis, in calories/cm^2/hour. The calorie is the small calorie, or gramme-calorie, and is the heat required to raise 1g of water $1^{\circ}C$ in the interval from 15° to $16^{\circ}C$.

This term is contrary to the proposed metric system of units known as SI (see Anon, 1968) but is used to compare the energy of available light with the known calorific content of carbon assimilated by the alga (see Chapter 5 photosynthetic efficiences). The $15^{\circ}C$ calorie is equivalent to 4.1855 Joules so conversion from cal/cm^2/hour to J/m^2/s (SI) is given by the following factor

$$\frac{x.\ 10,000}{4.1855.60.60} = x.\ 0.664\ \ J/m^2/s$$

where x is in cal/cm^2/h

In some cases the light intensities measured were compared with data of Kain (1966), who has used the term μg cal/cm^2/sec and the conversion factor from (g) cal/cm^2/h to μg cal/cm^2/s is

$$\frac{x.1,000,000}{3600} = x.277.8\ \mu g\ cal/cm^2/s$$

where x is in cal /g cal /cm^2/h.

Data in cal/cm^2/h is converted to the illuminance units, lux and foot-candles using the following factor from Spence & Chrystal (1970).

1 cal/cm^2/h = 2224 lux (Spence & Chrystal, 1970 mean of 6).

= 206 fc (where 1fc = 10.8 lux).

Some other conversion factors are:

$$1 \text{ cal/cm}^2\text{/h} = 1.16 \times 10^3 \text{ ub/cm}^2$$
$$= 11.63 \text{ W/m}^2$$
$$= 1.16 \times 10^4 \text{ erg/cm}^2\text{/s}$$
$$= 1.0 \text{ langley /h}$$

1. Light irradiance during 'in situ' experiments; the integrating photometer

The relationship between solar radiation and photosynthesis in the sea has been reviewed by Holmes (1957), Strickland (1958) and Levring (1966). The available light irradiance in the sea at any particular locality will depend upon the amount of light energy reaching the surface of the sea and the subsequent effect of the sea on this light.

The irradiance at sea level will depend on the solar altitude, the latitude of the site, the distribution of clouds, and the reflectivity of the surface of the sea. These factors show diurnal and seasonal variations so instantaneous recordings of total light energy are of limited use in comparing data from different times and localities. Thus over the course of a few hours during the 'in situ' experiments surface irradiance undergoes considerable change even on cloudless days.

The light irradiance actually penetrating the sea is attenuated by absorption and scattering and the light at any one depth at one particular wave-length is described by the equation,

$$I_x = I_o \cdot e^{-kx}$$

(Atkins et al., 1938)

where k = the diffuse attenuation coefficient
Ix = light irradiance at depth x
I_o = light irradiance at surface

The diffuse extinction coefficient is characteristic of waters and has different values for different wavelengths. The spectral composition of light changes with depth. Tyler (1959), Smith & Tyler (1967), have shown that natural waters are very efficient monochromators. Jerlov (1951) has analysed and classified water types from the very clear Oceanic Type I through Oceanic Type II

and finally the least clear Oceanic Type III. Jerlov (loc cit) has also defined 9 coastal types. The change in spectral composition from the blue-green wavelength maximum of about 480 nm in clear ocean water to the yellow-green maximum of about 550 nm of coastal waters has been explained by Kalle (1951, 1966) and Jerlov (1964) as being due to the increased amounts of vegetable decay products (humus), suspended matter and phytoplankton which shifts the spectral transmission to longer wavelengths.

The preferential absorption of long wavelength (red) light and this high absorption of short wavelength light in the visible spectrum means that coastal waters are predominantly green in colour and have a transmission maximum at about 550nm (Levring, 1966).

The fluctuation in irradiance and spectral composition described means that measurement of light in connection with productivity experiments requires either a knowledge of the diffuse extinction coefficient or, as was used in this study, an integrating photometric device.

The integrating photometer has been described in more detail by Drew (1972b). The instrument is shown in Figure 2:7 and consists of a selenium photovoltaic cell (3) which acts as a cosine collector of light irradiance and whose sensitivity depends on the surface area of collection. The output from the cell is passed to a mercury coulometer (5), the column of which is broken by a small bubble of electrolyte solution. When a current is passed through the coulometer the 'gap' is displaced towards the positive electrode. Gap movement down to 0.2mm can be detected by eye so that 1% of full gap movement is easily measured. At the low resistance (20 ohms) of the coulometer the response of the selenium photocell is linear to incident irradiance so the gap movement, being directly proportional to current flow, is directly proportional to incident light irradiance.

Operation, treatment of results and calibration of the instrument.

The photometer was switched on at the beginning of 'in situ' experiments by turning the polarity switch (4) towards whichever side had the longest path of mercury in the coulometer; the 'trailing' mercury meniscus was recorded

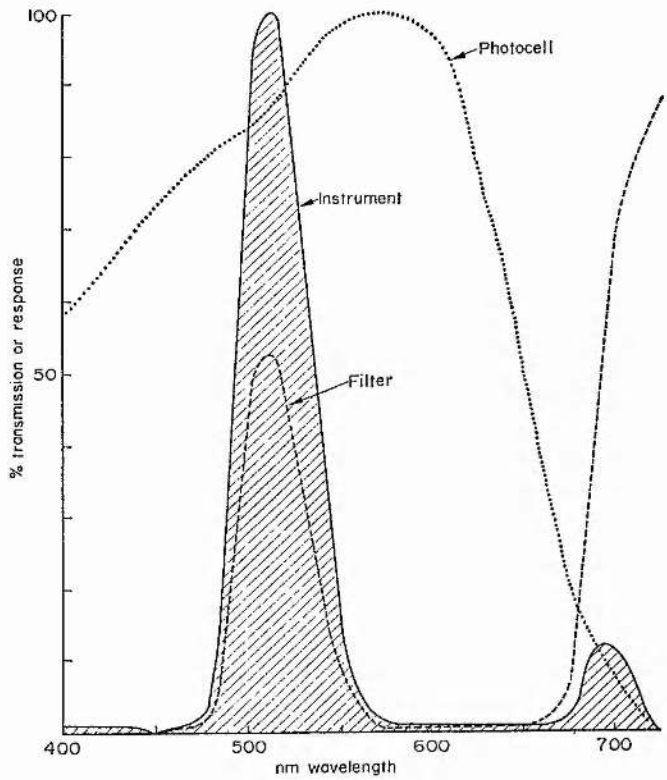


Fig. 2. Spectral characteristics of instrument and components.

The transmission of the green cinemoid filter, the spectral response of the photocell, and a combination of these to give the instrument's spectral response are shown in the above figure (reproduced by kind permission of Dr. E. Drew, from Drew, 1972b).

The value of gap movement recorded by the surface photometer was reduced by 7.5% to correct for the red response due to filter transmission beyond 650 nm. This correction is not necessary for the submerged instruments since red light is rapidly absorbed in water.

before the dive and the direction of movement required was noted on the data sheets. The movement of the gap is completely reversible but the gap must not be allowed to reach the end of the coulometer as the circuit was completed and the instrument could not be used again until another coulometer was fitted.

Since underwater light is predominantly green (see above) light of wavelength about 510nm was measured by this instrument with the aid of a green cinemoid filter (2) interposed between the opal perspex cosine collector (1) and the selenium photovoltaic cell (3).

In the 'in situ' experiments a surface photometer and one at each depth or habitat station was used. Various corrections had to be made to convert the readings from these instruments to absolute light energies. Firstly the surface photometers were corrected for the transmission of red light beyond 650nm (7.5% correction)*; this was unnecessary for the submerged instruments as red light is rapidly absorbed in the first few metres (see above). The surface photometer readings were also corrected for the different response of cosine light collectors in air and water; a correction of minus 25% was applied by Smith (1969) but in this case an overall correction of minus 20% (including the aforementioned 7.5%) was applied.

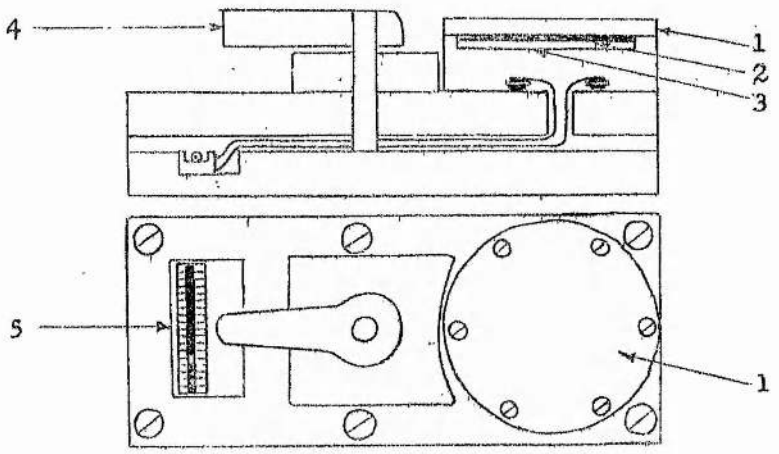


Figure 2:7 Integrating photometer.

The above information and diagram were kindly made available to me from proofs of the paper by Drew (1972b). * See opposite page.

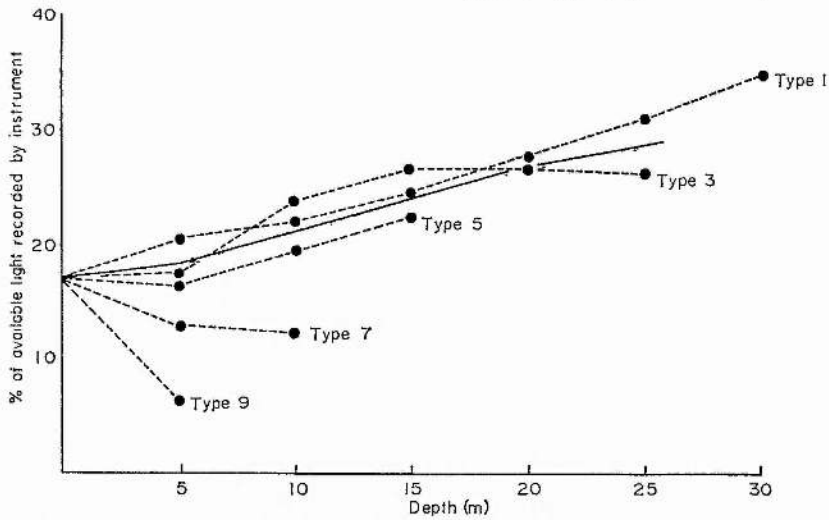


Fig. 4. Proportion of total incident light energy to which the integrator responds at various depths in waters of various clarities. ---, Computed values for each type of water; ——, average for Jerlov's waters of Coastal Types 1–5.

The above figure is reproduced from Drew (1972 b). The proportions of total light energy to which the instrument, with its green filter, should respond at different depths in waters of different clarities are shown and were calculated from the spectral distribution of incident light energy at each depth (data of Jerlov, 1951) and the spectral sensitivity of the instrument. The percent responses of the instrument at given depths in waters of Jerlov's Coastal Types 1 to 5 are very similar and average values are shown as a heavy line. The correction factors outlined in Table 2:6 and others used to convert the response of the instrument to total light energy were read from this line for the various depth stations where light energy was measured. Thus it was not necessary to account for different water Types and waters of greater opacities than Type 5 were not considered as no significant gap movements were recorded under these conditions.

The values from an experiment in West Scotland (Experiment B, Chapter 5) are plotted in Figure 2:8 as percentages of the corrected surface values and this attenuation curve shows a logarithmic attentuation with depth and also corresponds to the coastal water Type 4 (Jerlov, 1951) as expected for this area.

The response of the underwater photometers had to be corrected for the spectral sensitivity of the instrument and the proportions of total light irradiance to which this instrument should respond at different depths are set out in a correction curve in Drew (1972, loc cit). The most commonly used correction factors for the depths at which experiments and light measurements were carried out are set out in Table 2:6. See opposite page for further details.

Table 2:6

Integration response correction factors

Depth	Correction factor
3.1	100/18
10.7	100/22
18.3	100/26.5

The values for corrected gap movements on land showed that 1mm moved was equivalent to 1 cal/cm^2 but underwater the response is somewhat reduced and, in this case, 1 cal/cm^2 was indicated by a corrected gap movement of only 0.8 mm so the corrected gap movement was divided by this factor to give the final absolute light irradiance in cal/cm^2/h.

2. Light irradiances in the laboratory

Various light sources were used for experiments on photosynthesis, metabolism, translocation, and for culturing Laminaria hyperborea sprorophytes. The light irradiance used in these experiments were measured with an I.S.C.O. Spectroradiometer, using either a Model SRR Programmed Scanning Recorder or reading directly from the radiometer.

A continuous scan of visible light irradiances was obtained from 400 nm to 750 nm with either the direct head of the instrument below the light source or, for more inaccessible places, a 2 m fibre-optic remote probe

terminating in a teflon diffusing screen. The diffusing screen was held horizontally under the lamp whilst measuring irradiance.

Bandwidths of 25 nm intervals were measured and the total irradiance from 400 nm to 750 nm was integrated either by summation of the direct readings or mathematically from the area under the recorder plot. Calibration factors were applied to each 25 nm waveband and the total obtained in μWatts/cm^2. This was converted to cal/cm^2/h using the following factor:

$$1\ \mu W/cm^2 = 8.604 \times 10^{-4}\ cal/cm^2/h$$

(Šesták, et al. 1971).

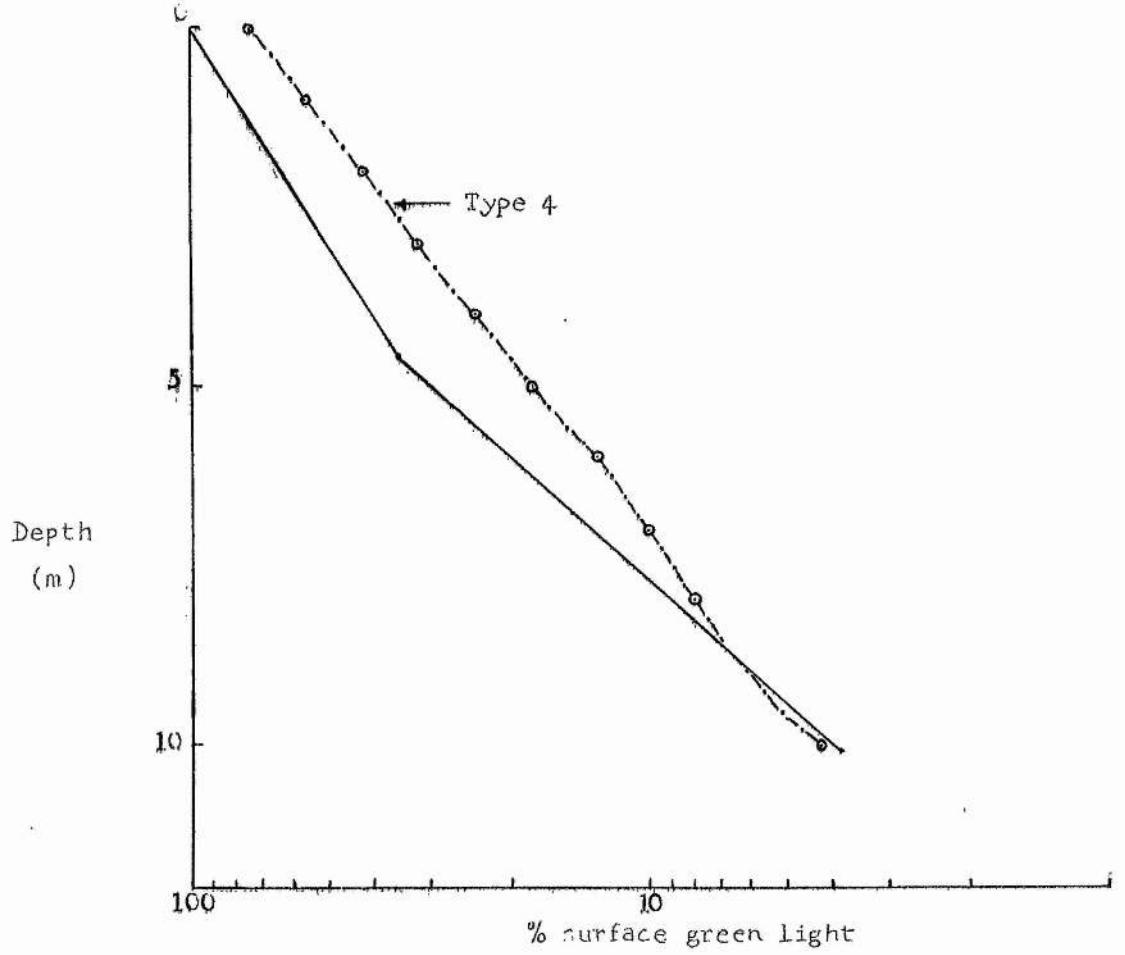


Figure 2:8 Attenuation curve from integrator data of experiment B, Chapter 5.

CHAPTER THREE

BIOMETRIC DATA

Introduction

There have been extensive surveys of the quantities of sublittoral brown algae of commercial importance. Thus both the Scottish Seaweed Research Association and the Norwegian Institute of Seaweed Research have amassed a wealth of data on the densities and biomass of sublittoral Laminaria species (Annual Reports, S.S.R.A., 1945-1958; Grenager, 1953, 1954, 1955; Walker 1947, 1948, 1950, 1952, 1954a,b,c, 1955, 1956a,b, 1958; Walker & Richardson 1955, 1956, 1957a,b). Walker (1958) has summarised the data of ecological interest from these surveys. Boney (1965), Scagel (1966), and Chapman (1970) have reviewed aspects of the biology and quantitative ecology of sublittoral macrophytes.

A survey of sublittoral algae was first carried out during the 1939-45 war using grapnels and echo-sounders (Chapman, 1944). The Scottish Seaweed Research Association developed sampling techniques such as a view box combined with a spring grab to assess sublittoral seaweed quantities (Walker, 1947). These data, whilst assessing harvestable amounts of seaweed, are subject to large sampling errors. Thus spring grabs are now known to collect only 30 to 65% of their full sampling capacity on rocky substrates and they do not include the lower portions of the plant (Lüning, 1969b).

There have been only a few accurate and direct studies of biomass and productivity in sublittoral macrophytes. Aleem (1956) and McFarland & Prescott (1959), using SCUBA techniques, obtained more accurate estimates of the biomass of Macrocystis pyrifera. Bellamy & Whittick (1968a,b), Bellamy et al. (1968), John (1968, 1969, 1971), and Whittick (1969) have obtained primary production data for Laminaria hyperborea and Laminaria ochroleuca using increment quadrat cropping techniques. Lüning (1969b) has obtained standing crop and productivity data for L. hyperborea using SCUBA.

'In situ' cropping techniques have been used in the present study to obtain biomass and productivity data for a L. hyperborea forest in W. Scotland. The increment cropping technique was used to obtain a seasonal maximum lamina biomass and the productivity of the perennial parts (stipe and holdfast) was estimated from biomass/age relationships (Bellamy & Holland, 1966; John, 1968, 1971; Whittick, 1969). In this chapter the biometric data collected from Arisaig, Inverness-shire (56° 57'N; 05° 52'W) has been used for the following purposes:-

(1) to investigate the effect of depth on the growth of the alga

(2) to investigate the effect of the canopy on the growth of young plants

(3) to investigate seasonal aspects of growth and to obtain a value for net annual productivity and net assimilation rate.

There were 10 quadrats ($0.5m^2$), containing 218 plants, taken from 3.1m and 12 quadrats, containing 105 plants, from 9.1m. The quadrats were obtained over a period of 15 months. Data include the mean and standard error.

Results

1. Estimation of organic matter and calorific content of dry tissue

The organic matter content, or ash-free dry weight, for 2 January plants was estimated by bomb calorimetry. The calorific content of the tissues was also determined and these data are presented in Table 3:1.

Table 3:1

Ash, organic, and calorific content of tissues of L. hyperborea from 3.1m and 9.1m

Tissue	Ash content % dry weight		Organic matter % dry weight		Calorific content kcal/ g organic matter	
	3.1m	9.1m	3.1m	9.1m	3.1m	9.1m
Lamina	19.8	12.1	80.2	87.9	4.76	4.97
Stipe	28.5	24.8	71.5	75.2	4.55	4.35
Holdfast	33.2	26.4	66.8	73.6	4.49	4.62

The values from this Table, which are very similar to those from a larger number of plants analysed by Robertson (1970), have been used to convert

productivity and carbon fixation data to organic and calorific equivalents throughout this thesis.

2. The effect of depth on the growth of L. hyperborea.

Biomass data from two depths

The mean biomass and plant density found at each depth is shown in Table 3:2. This data was calculated from all the quadrats analysed disregarding seasonal changes.

Table 3:2

Mean biomass at two depths

Depth (m)	Fresh weight (kg/m^2)	Dry Weight (kg/m^2)	Density ($plants/m^2$)
3.1	20.4	3.4	22
9.1	7.7	1.2	9

The biomass at 9.1m was some 37% of that at 3.1m and the density was similarly reduced. The maximum site biomass found in this study is shown in Table 3:3.

Table 3:3

Maximum quadrat biomass at two depths

Depth (m)	Fresh weight (kg/m^2)	Dry weight (kg/m^2)	Organic matter* (kg/m^2)	Date
3.1	36.7	5.8	4.7	20.3.69
9.1	11.3	1.7	1.5	20.3.69

*Obtained using factors from Table 3:1.

Individual plant performance

The following parameters have been investigated to assess individual plant performance at the two depths:

Fresh weight holdfast (FWH in g), dry weight holdfast (DWH in g). Fresh weight stipe (FWS in g), dry weight stipe (DWS in g), length stipe (LS in cm), fresh weight old lamina (FWLi in g), dry weight old lamina (DWLii in g), fresh weight new lamina (FWLii in g), dry weight new lamina (DWLii in g) Area old lamina (ALi in cm^2), area new

lamina (ALii in cm^2). Specific lamina area for old lamina (SLAi in cm^2/mg dry wt.); and new lamina (SLAii in cm^2/mg dry wt). The mean value per plant for each parameter in quadrats from June samples is set out in Table 3:4.

Table 3:4

Individual plant performance (12.6.69)

Depth	n	DWH	DWS	LS	DWLi	DWLii	ALi	ALii
3.1	34	14.7±4.6	31.9±9.1	38.7±8.5	0.6±0.1	31.5±11.6	66.3±5.4	1850.2±494
9.1	12	12.8±4.4	27.9±10	50.3±10.4	2.5±0.7	29.4±10.9	144 ±31.5	1855±676

Depth	SLAi	SLAii
3.1	0.12±0.01	0.14±0.01
9.1	0.11±0.02	0.12±0.01

n - number of plants in each $0.5m^2$ quadrat

None of the differences between the mean values are significant at the 5% probability level of t, except ALi. It is apparent that the individual plant growth is not significantly reduced with depth. The overall mean plant dry weight for June quadrats and two obtained in January are shown in Table 3:5.

Table 3:5

Mean plant dry weight at two depths

Depth	January	June
3.1	93.8 ± 21.5	75.5 ± 22.4
9.1	87.0 ± 24.5	70.6 ± 25.4

It can be seen that the individual plant biomass is not significantly reduced with depth. However, this is not always the case, and Table 3:6 shows that individual plant performance may sometimes be reduced at depth.

Table 3:6

Individual plant performance (7.6.70)

mean	n	DWH	DWS	LS	DWL	ALii	SLA(DW)
3.1	6	$19.5^{+}_{-}5$	$82.2^{+}_{-}10.3$	$134.7^{+}_{-}5.4$	$60^{+}_{-}11.4$	$4516.7^{+}_{-}700.2$	$0,081^{+}_{-}0,006$
9.1	5	$4.4^{+}_{-}1.5$	$10.4^{+}_{-}3.6$	$10.4^{+}_{-}3.6$	$9.2^{+}_{-}5.2$	$1175^{+}_{-}614$	$0,134^{+}_{-}0.009$
		*	**	**	*	*	**

mean	MEAN DW
3.1	$161.7^{+}_{-}24.5$
9.1	$24.2^{+}_{-}9.0$
	**

* - denotes significant difference at p = 0,05

** - denotes significant difference at p = 0,01

Maximum plant performance at two depths

The maximum value for each growth parameter found at the two depths is given in Table 3:7 together with the date of collection of the sample.

Table 3:7

Maximum data for growth parameters at two depths

D.	FWH	DWH	FWS	DWS	LS	FWLi	DWLi	FWLii
3.1	419,8- 19.10.69	108.7 12.6.69	1078 10.1.70	284.2 20.3.69	183 10.1.70	712 19.3.69	139 10.1.70	954.5 12.6.69
9.1	260.3 19.10.69	71 19.10.69	910 20.3.69	138.3 20.3.69	133.5 20.3.69	327 20.3.69	51.7 20.3.69	877.2 11.5.69

D.	DWLii	ALi	ALii	SLAi	SLAii
3.1	328.1 12.6.69	5575 19.3.69	8500 12.6.69	1.00 12.6.69	0.40 12.6.69
9.1	107.6 19.10.69	3025 20.3.69	11375 11.5.69	0.16 12,6.69	0.20 20.3.69

Frequency distribution of stipe lengths

The frequency distribution of stipe lengths in the two depth populations are plotted as histograms in Figure 3:1.

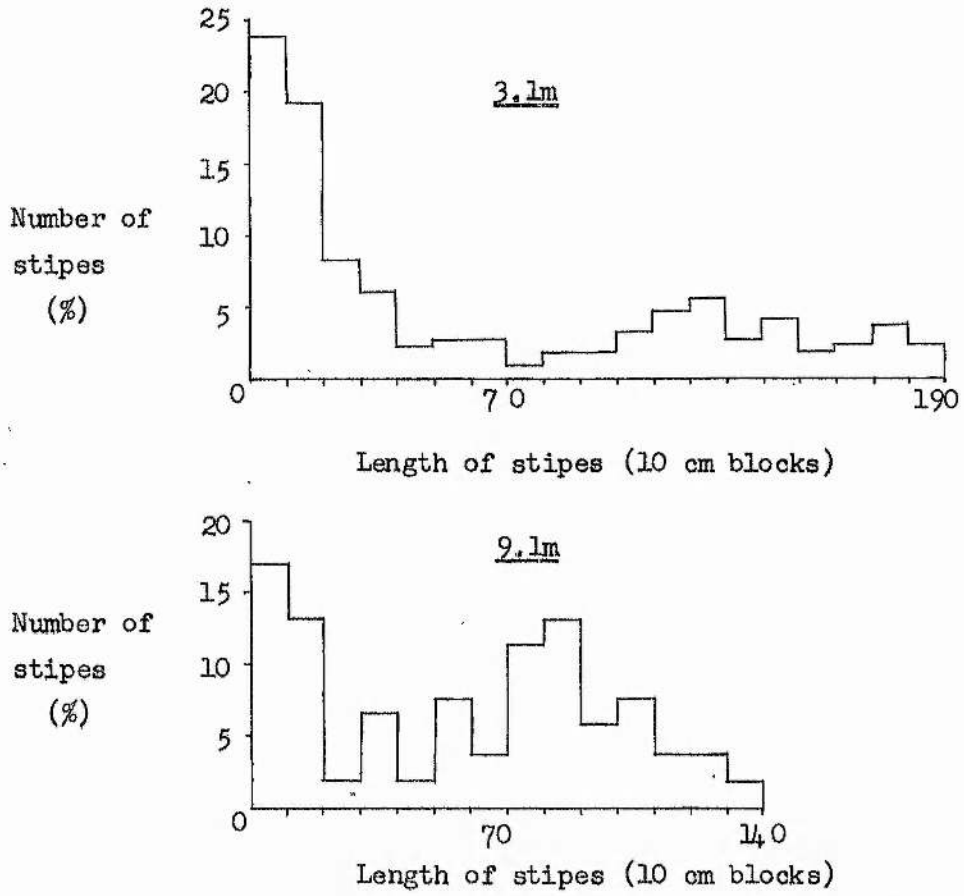


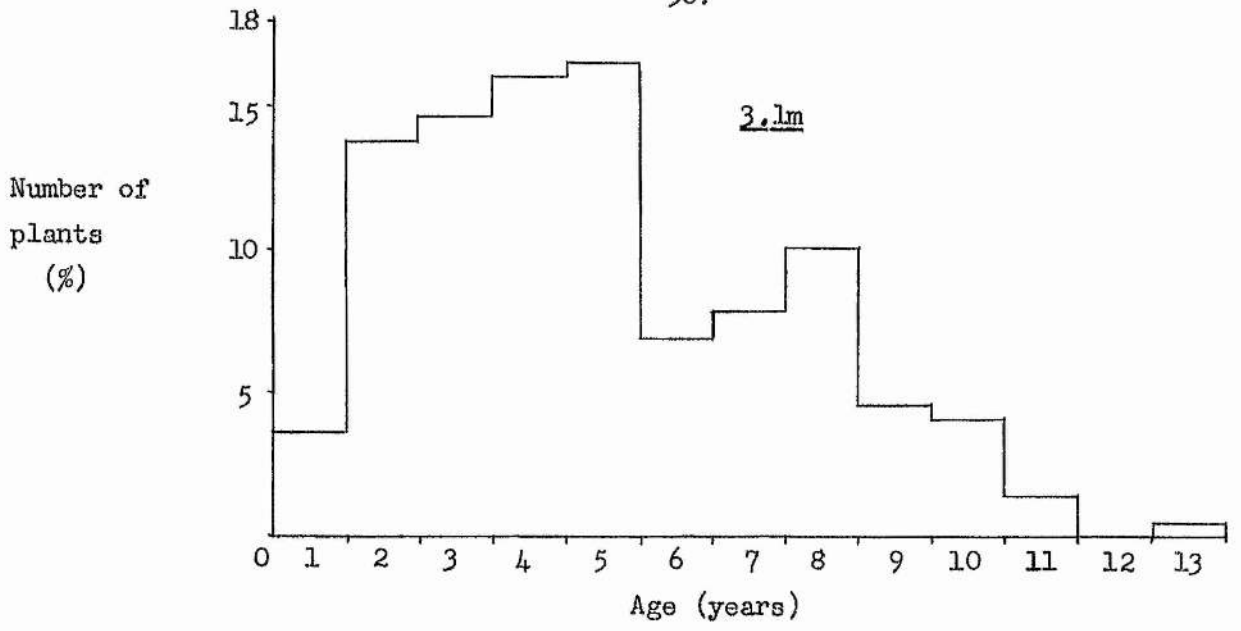
Figure 3:1 Frequency distribution of stipe lengths at two depths

The data are not treated for possible age structure imbalance as was done by Kain (1963), and the values in each 10cm block are percentages of the total number of plants found in all the quadrats at each depth. The lack of plants with intermediate lengths of stipe is evident in both the shallow population and the deep population.

Frequency distribution of ages at two depths

The age structures of the two populations at Arisaig are shown as frequency distribution histograms in Figure 3:2. The data suggest that the populations in shallow water (3.1m) exist under much the same environmental conditions as the Isle of Man populations described by Kain (1963).

The population at 9.1m, however, shows a peak in 6 year plants which probably reflects favourable establishment conditions.



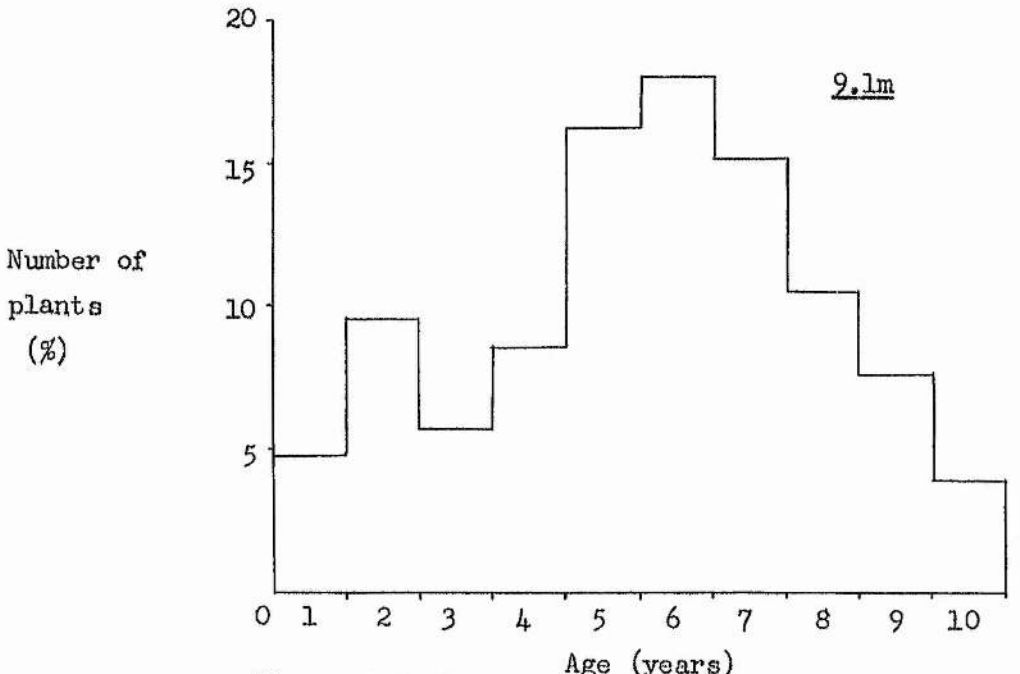


Figure 3:2 Frequency distribution of ages at two depths.

Leaf Area Index

The Leaf Area Index (LAI) describes the size of the photosynthetic apparatus of the plants present in a given area (Sěsták et al. 1971), and is defined as the leaf area per unit ground area. In the case of L. hyperborea it is the total m^2 of lamina area per unit m^2 sea-bed area. The mean and maximum values for LAI recorded are given in Table 3:8.

Table 3:8
Mean and maximum LAI (m^2 lamina/m^2 sea-bed)

Depth	Mean	Maximum	
3.1	7.3	12.8	(June)
9.1	3.4	4.5	(June)

The mean value does not take into account the marked seasonal variation in lamina area (see section 4). LAI changes with the seasonal expansion of lamina area and with the competition for light in the forest canopy. The values of LAI for canopy plants only are given in Table 3:9. The canopy plants are considered to consist of all plants from 7 to 10 years (see section 3) and the data in Table 3:9 are for the new lamina only. The data shown are the LAI's for the months when quadrats were sampled. An average figure from January to October and a summer value from June to October are also given.

Table 3:9

Depth and seasonal variation in LAI for new lamina of canopy plants; 7 to 10 years

(m) Depth	January	March	May	June	October	mean Growing season LAI January-October	mean Summer LAI June-October
3.1	0.03	1.67	5.57	7.52	4.48	3.85	6.0
9.1	-	0.93	3.02	1.96	1.75	1.92	1.86

It can be seen that LAI decreases with depth and changes with the growing season as the individual plant lamina expands. The above data is for the canopy layer of plants (7 to 10 years) and has been used in Section 4 to obtain values for the net assimilation rate of these plants so that a comparison between the productivity estimate from biometric data and that from C14 data from chapter 5 could be made. In Table 3:10 the LAI using all the plants in a quadrat is given. The values are the 'summer LAI' i.e. the mean of the values from the June and October quadrats.

Table 3:10

Summer LAI (June/October) using all plants in quadrat

Depth	LAI (m^2 lamina/m^2 sea-bed)
3.1	7.21
9.1	3.27

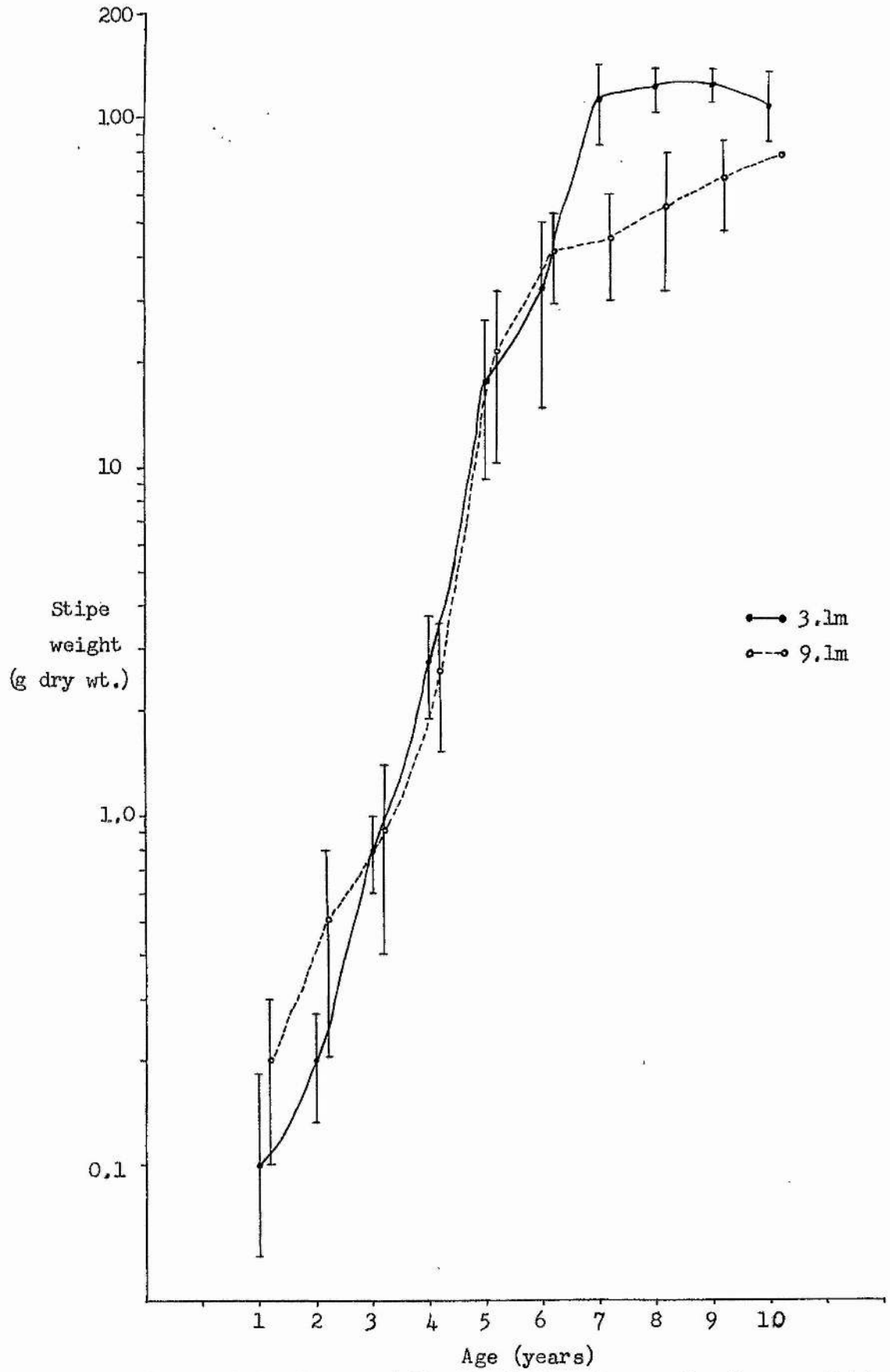


Figure 3:3 Stipe weights at different ages for the populations at 3.1m and 9.1m

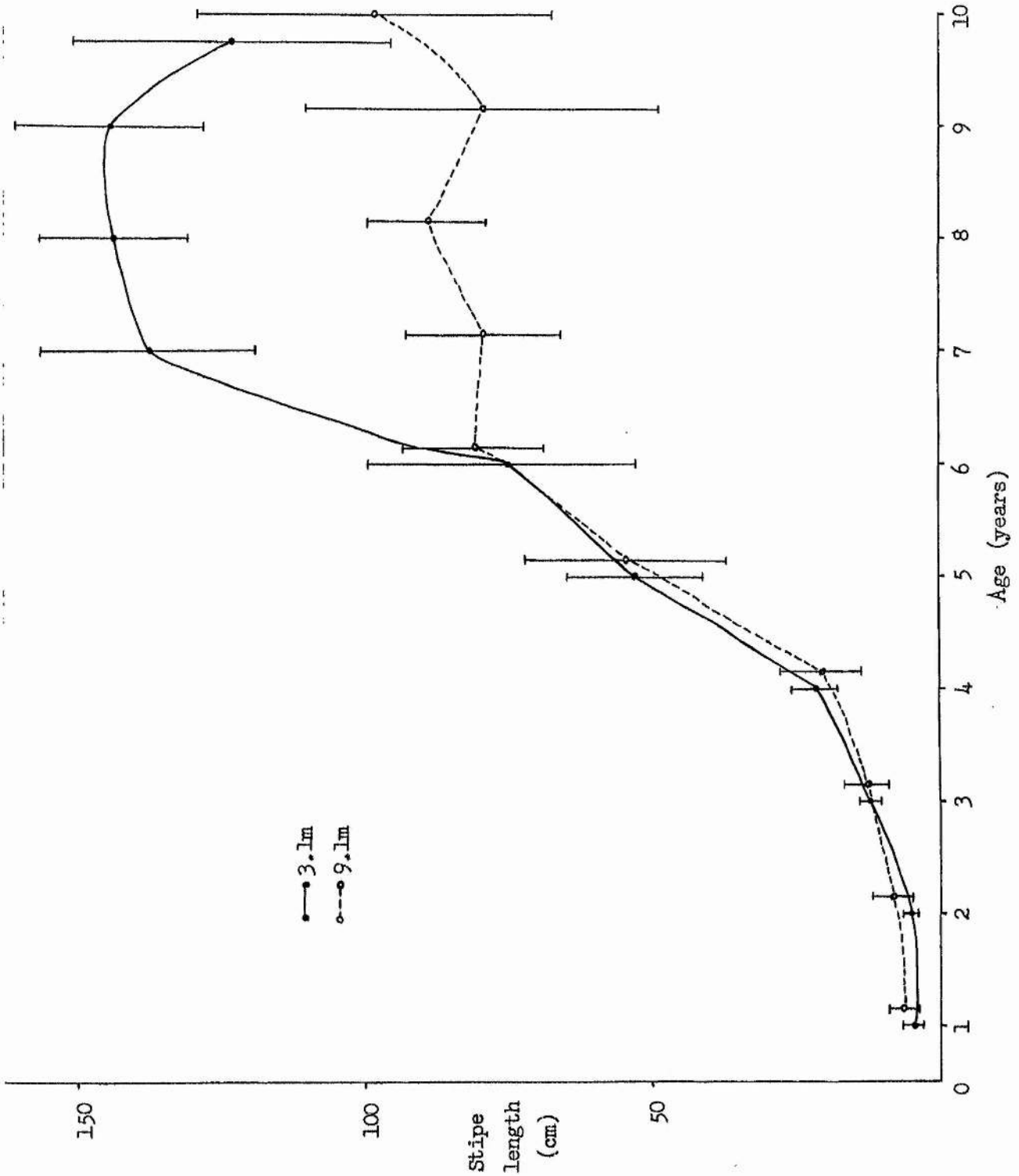


Figure 3:4 Stipe lengths at different ages for the populations at 3.1m and 9.1m.

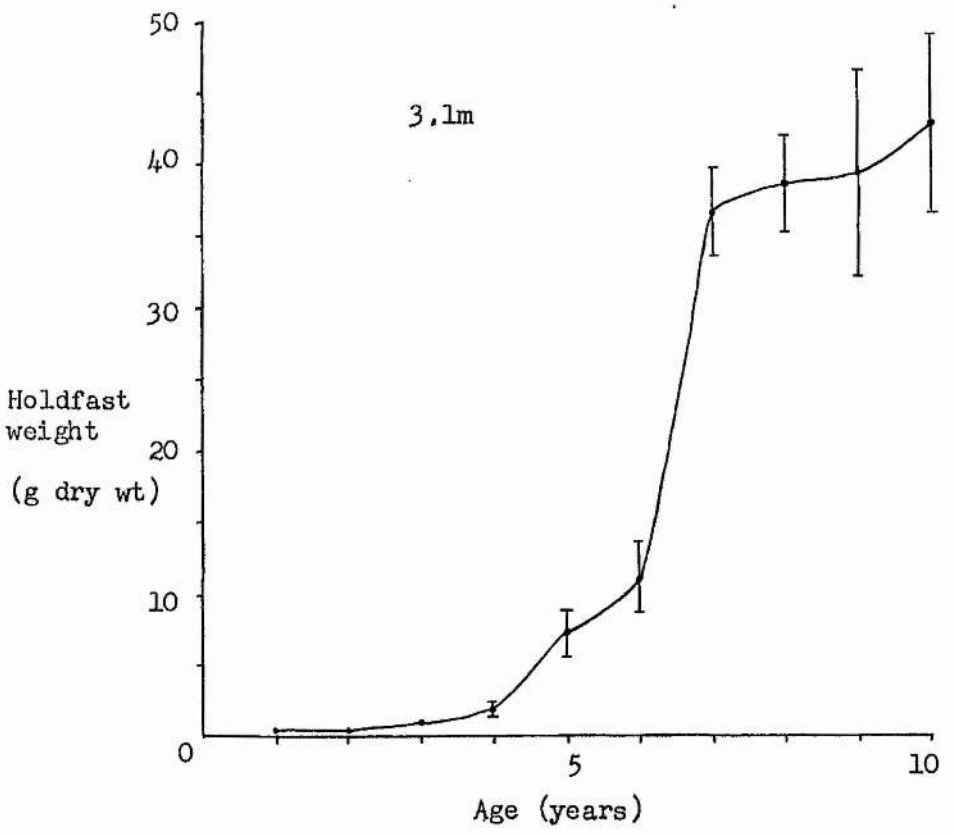
The canopy plants (7 to 10 years) make up 83% of the total LAI at 3.1m but only 50% of the LAI at 9.1m. This is a reflection of the reduced density at depth and means there is considerably less competition for light and much less of a canopy layer of older L. hyperborea plants at 9.1m than at 3.1m.

3. Biomass and age relationships (life span data) and canopy effects

There have been numerous studies of changes in the growth of L. hyperborea with age (Black et al. 1959; Kain, 1963, 1967, 1971b; John 1968; Whittick, 1969; Bellamy & Whittick, 1968a; Larkum, 1972). The present data are used to show changes during the life span of the plant and the effects of the canopy on the growth of young plants. The data for the perennial parts (stipe and holdfast) are used to obtain productivity estimates. Figure 3:3 shows the mean plant stipe weight plotted on logarithmic scale against age with 95% confidence limits. This shows the data for young plants more clearly and it can be seen that the stipe weights of 1 to 3 year plants at 3.1m are less than similarly aged plants at 9.1m. Old plants at 9.1m have a significantly reduced stipe weight compared to their counter parts at 3.1m.

It can also be seen that the stipe weight at 3.1m levels off after 7 years and the group of plants from 7 to 10 years is held to constitute the 'canopy layer' in the forest. Stipe weight at 3.1m actually drops after 9 years, whereas at 9.1m growth continues to rise up to at least 10 years. This suggests some factor(s) is acting on the older shallow plants which is absent at depth.

In Figure 3:4 the stipe length is shown for each age group at the two depths. A similar pattern to stipe weight is apparent. Thus young plants at 9.1m grow slightly better than their counterparts in the dense forest at 3.1m but older plants at 9.1m show a reduced growth compared with the same aged plants at 3.1m. It can be seen from this graph that plants undergo a period of slow growth up to about 4 years after which a period of rapid growth occurs.



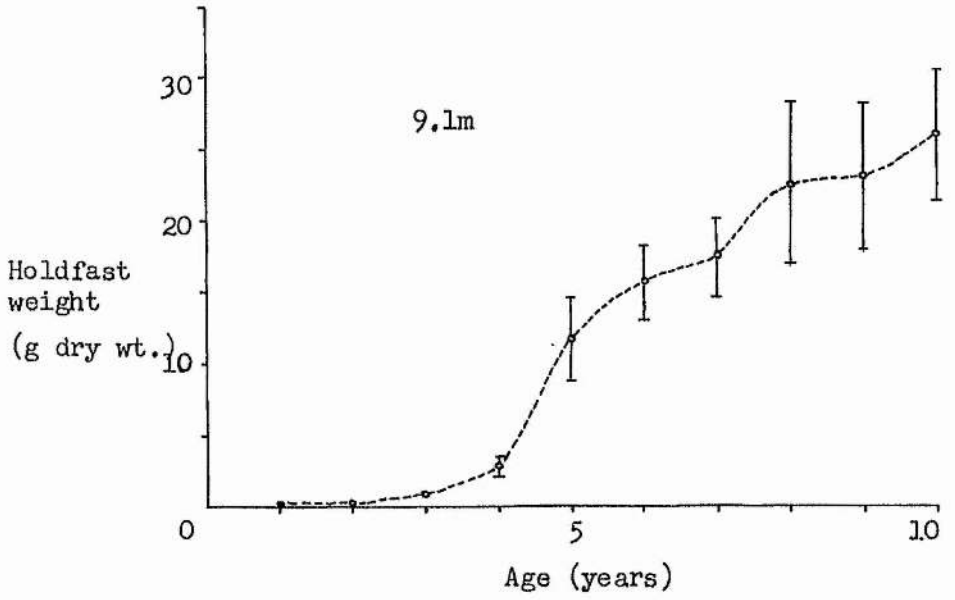


Figure 3:5 Holdfast weight at different ages for the populations at 3.1m and 9.1m.

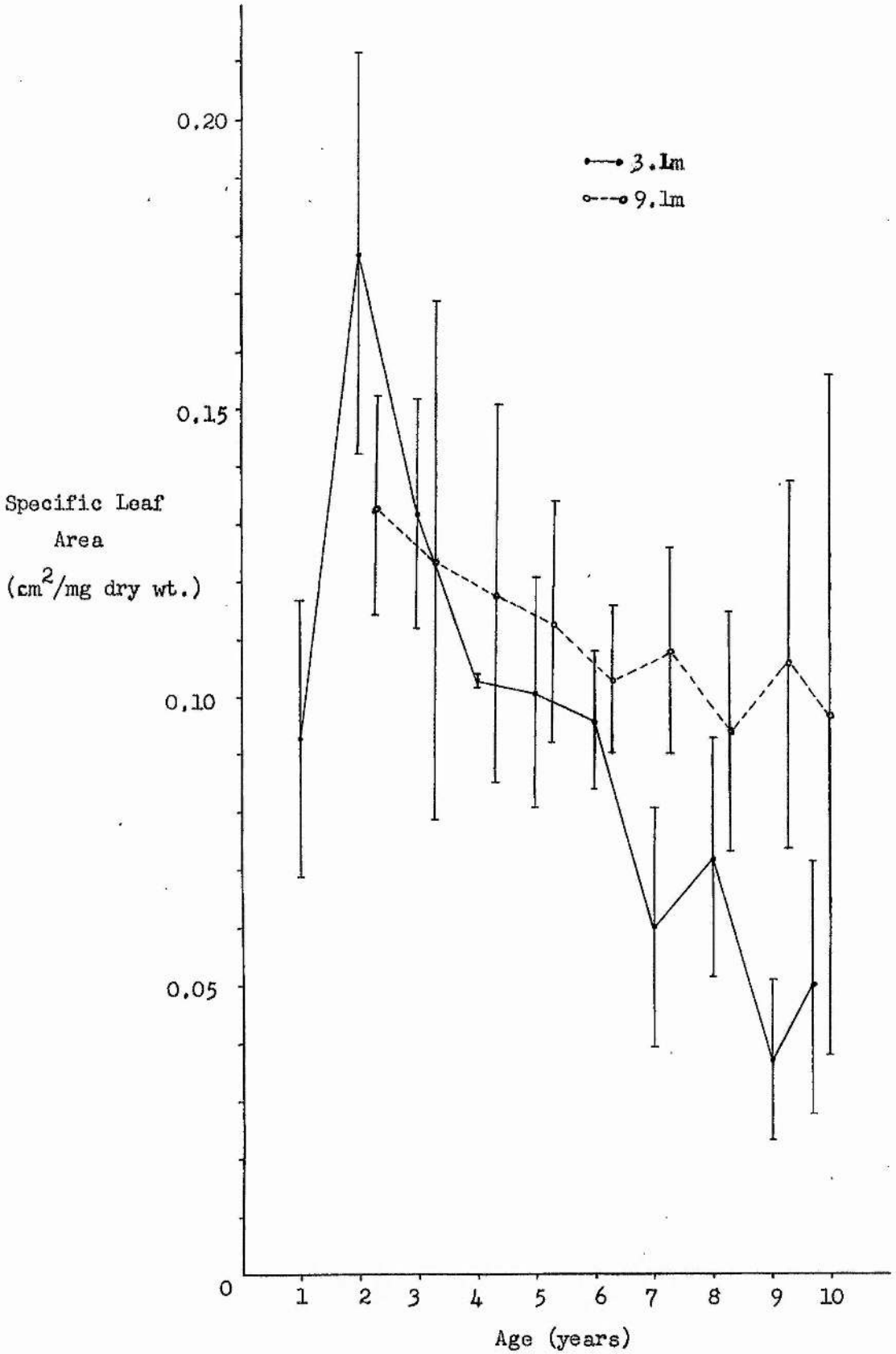


Figure 3:6 Specific leaf area at different ages for the populations at 3.1m and 9.1m.

The presence of a 'canopy layer' of plants from 7 to 10 years is evident at 3.1m and the absence of very tall 10 year plants there is noted.

The age at which rapid growth starts (4 years) is later than that quoted by Kain (1963), i.e. 2 years, and Whittick (1969), -who found that rapid growth started after 3 years. This may, in part, be due to the different aging methods used (see chapter 2). That the aging may not have been seriously inaccurate is indicated by the data of Kain (1963, 1967), who showed that longevity increased in more northerly populations. Kain (1969) quoted populations at Longa Island, Gairloch (57^{o} 42.7'N; 05^{o} 40.5'W) having 13 year plants compared with Isle of Man populations where plants over 7 years are rare (Kain, 1967). The population studied at Arisaig with up to 10 year plants would fit this pattern of increased longevity in more northerly latitudes.

The relationship between the other perennial part of the plant, the holdfast, and age is shown in Figure 3:5. The sigmoidal shape of the growth curve at 3.1m illustrates the rapid growth phase from 4 to 7 years indicating that similar environmental factors are acting on the holdfasts as on the stipes during the life-span of the plant. Growth with age is more linear at 9.1m. The data from these graphs have been used in section 4 to assess the net annual production of the plant. Figure 3:6 shows the Specific Leaf Area (SLA) of different age groups at the two depths. This parameter - the lamina area (cm^2) per unit lamina weight (mg dry wt) - varies with the depth and density of the forest. This is indicated by the increased SLA of young plants (2 to 3 years) at 3.1m compared to young plants at 9.1m - the lamina is 'thinner' under the canopy - whilst in the upper age classes the situation is reversed and plants at 9.1m have thinner laminas than plants at 3.1m. It should be noted that there is a large variation in the data and that the only significant differences (i.e. with no overlap of 95% confidence intervals) were found in 7 and 9 year plants at the two depths.

4. Seasonal growth of L. hyperborea : net annual primary production

Harries (1932), Kain (1963), John (1968), Whittick (1969), and Lüning (1969a, 1970a), have described the seasonal mode of growth in L. hyperborea, with a period of rapid primary and secondary growth from January to about June. The rate of growth is drastically reduced and even reversed during the second half of the year.

Lamina biomass and area changes

Figure 3:7 shows the rapid expansion in lamina area during the first few months of the year. In this figure a feature termed relative lamina area is used - that is, lamina area ($cm^2/\frac{1}{2}\ m^2$)/ stipe dry weight + holdfast dryweight ($g/\frac{1}{2}\ m^2$) it shows that lamina area at 3.1m expands to a

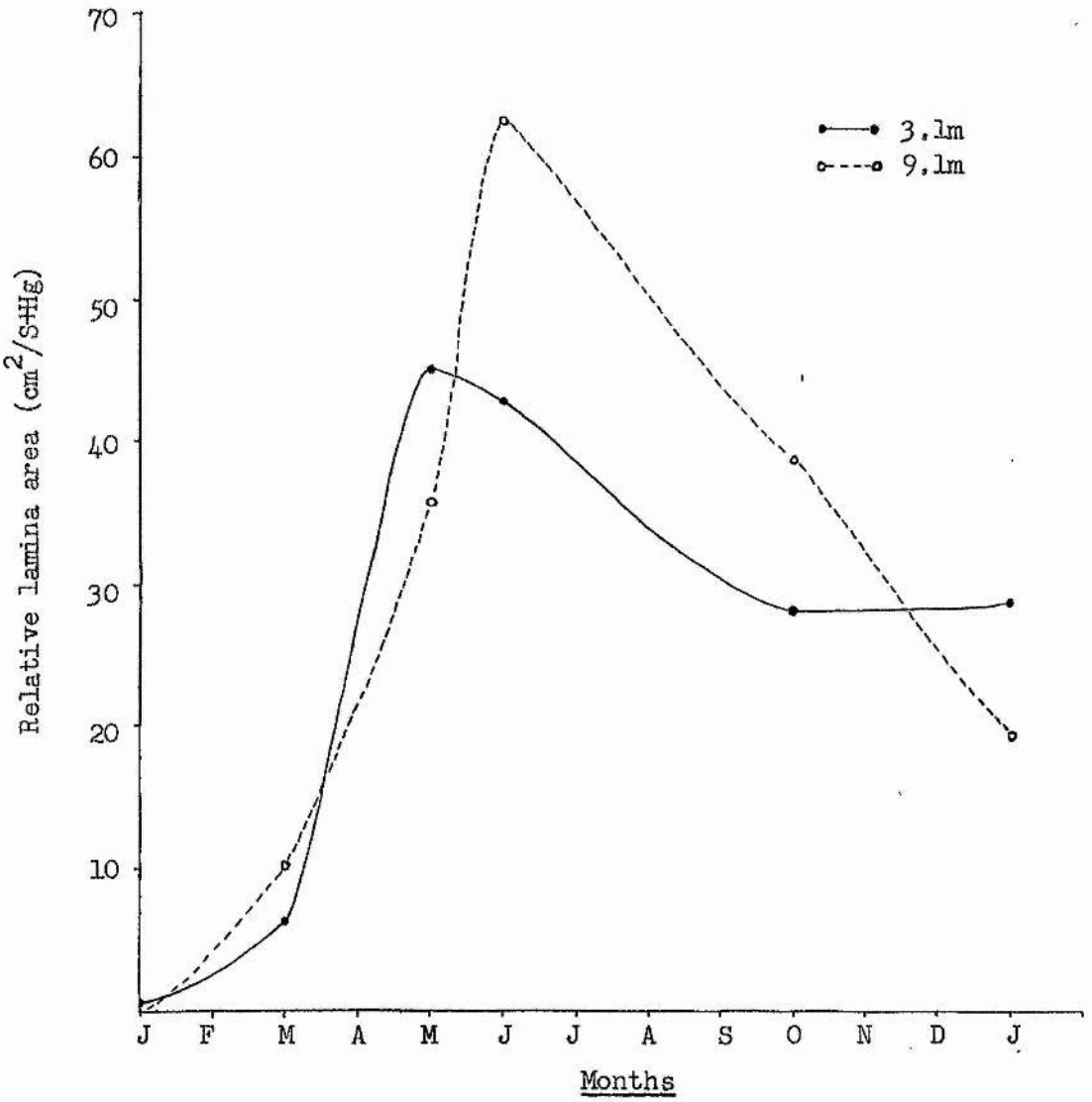


Figure 3:7 Seasonal variation in Relative Lamina Area of populations at 3.1m and 9.1m.

maximum in May, whilst 9.1m plants reach maximum expansion in June. Lüning (1970a), has reported similar depth differences in seasonal lamina growth and suggests that deeper plants lag behind shallower growing plants because underwater irradiance reaches optimum levels for growth at a later stage in deeper water than at shallower depths. An increased lamina area at depth is indicated (cf. SLA data at depth). However, the term relative lamina area also involved stipe and holdfast biomass which does show some seasonal changes.

Lamina area is not a very satisfactory parameter of growth since it may reflect changes in turgor status which obscure actual increments in cellular tissue. This can be seen when comparing growth as lamina dry weight expressed as a percentage of fresh weight with the absolute values of lamina dry weight during the seasonal growth. Thus Figure 3:8 shows that

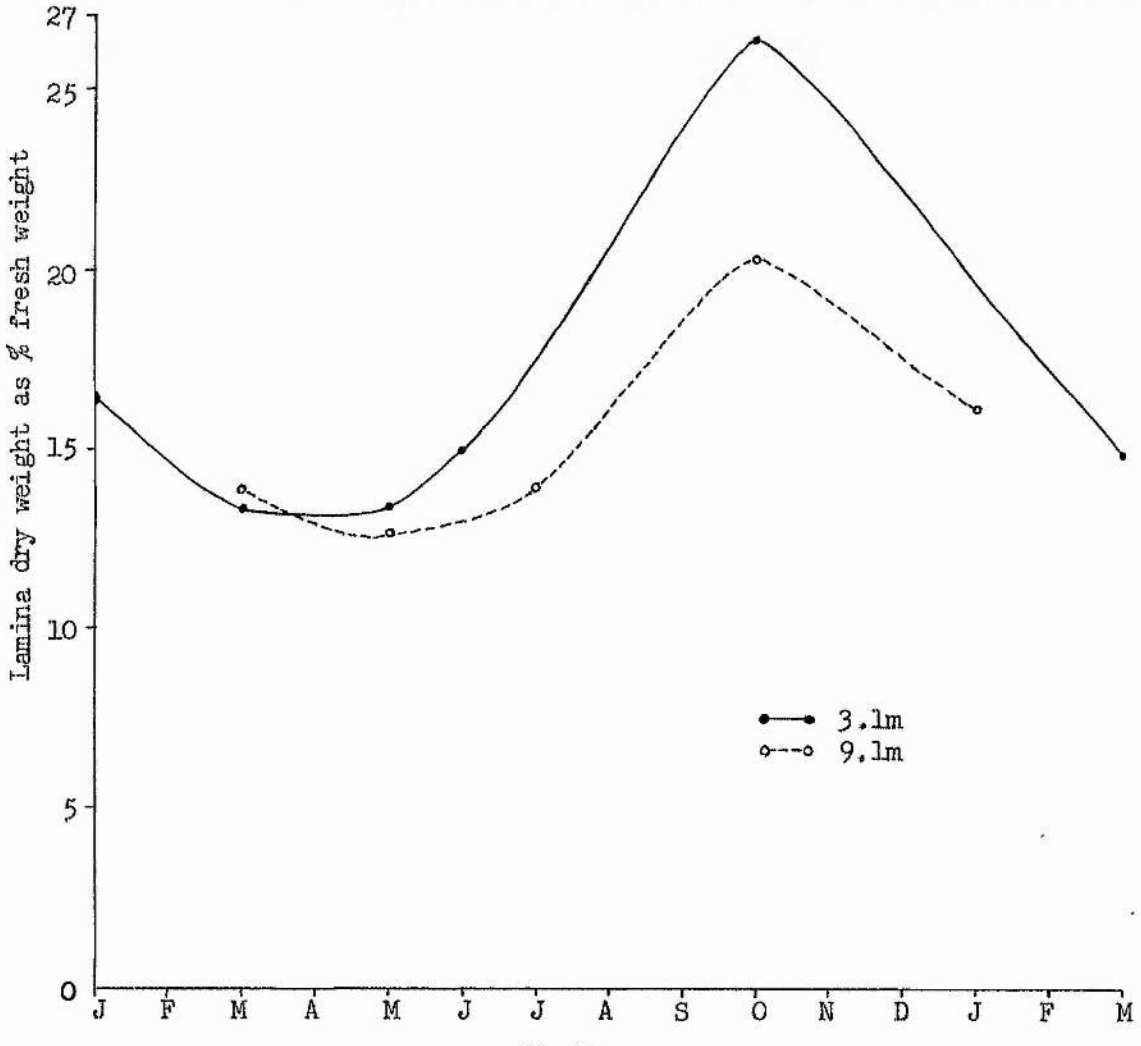


Figure 3:8 Seasonal variation in lamina dry weight (% fresh weight) at 3.1m and 9.1m.

from January to May a decrease in dry weight occurs when expressed as the percentage of fresh weight. However, when lamina growth is expressed as mean dry weight for the canopy plants (7 to 10 years; Figure 3:9) it can be seen that a continual increment in weight occurs from January to reach a seasonal peak in October. Figure 3:9 indicates that there are 3 phases in lamina growth during the season.

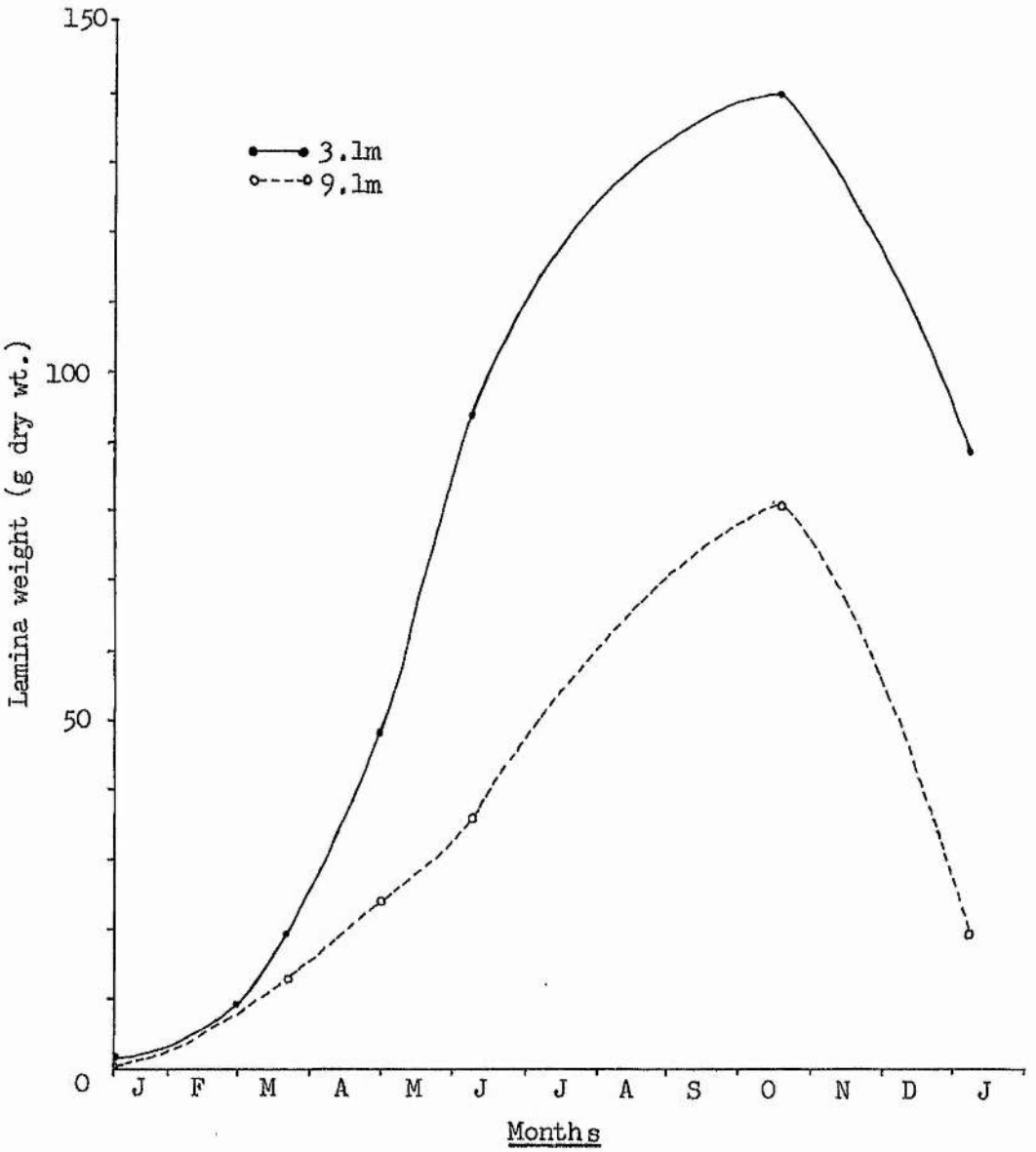


Figure 3:9 Seasonal variation in mean lamina dry weight for canopy plants (7 to 10 years) at 3.1m and 9.1m.

Phase 1 - The 'lag' phase.

This period of growth is characterised by a rather slow increase in dry weight and Figure 3:9 shows that from January to March the average canopy plant produces up to 9g dry weight of new lamina. Unfortunately there were not enough plants in each quadrat to analyse the lamina dry weight increment over this period for each age class.

The 'lag' phase lasts from January to March in plants from 3.1m, whilst it appears to be extended to May at 9.1m.

Phase 2 - The rapid growth phase

From Figure 3:9 it can be seen that a period of rapid lamina growth takes place from March to June at 3.1m. Deep growing plants appear to initiate this rapid growth in May.

Phase 3 - The slow growth phase.

This period of growth is characterised by a much slower growth rate up to the seasonal maximum lamina weight. It lasts, in plants at 3.1m from June until October, whilst at 9.1m there is a less sharp reduction in growth rate but a change in the slope of the graph is discernible after August.*

The factors concerned with this slowing up in growth are discussed in chapter 5.

Seasonal maximum lamina biomass

No samples were taken between June and October so that the seasonal maximum lamina biomass must be taken as the value in the October sample.

Annual growth of stipe and holdfast

The perennial parts of L. hyperborea do not show as dramatic a

* These dates are approximate since regular monthly quadrats were not taken.

seasonal variation as the lamina. Figure 3:10 shows that both stipe and holdfast biomass increases from January to March.

Stipe and holdfast biomass at 3.1m appears to level off after May, whereas at 9.1m an increase is found for holdfasts which reach a peak in October. Stipes at 9.1m do not exceed the March maximum value.

Net annual primary productivity

In this part an estimate has been made of the net annual primary productivity in L. hyperborea using the biomass increment cropping technique., and biomass relationships.

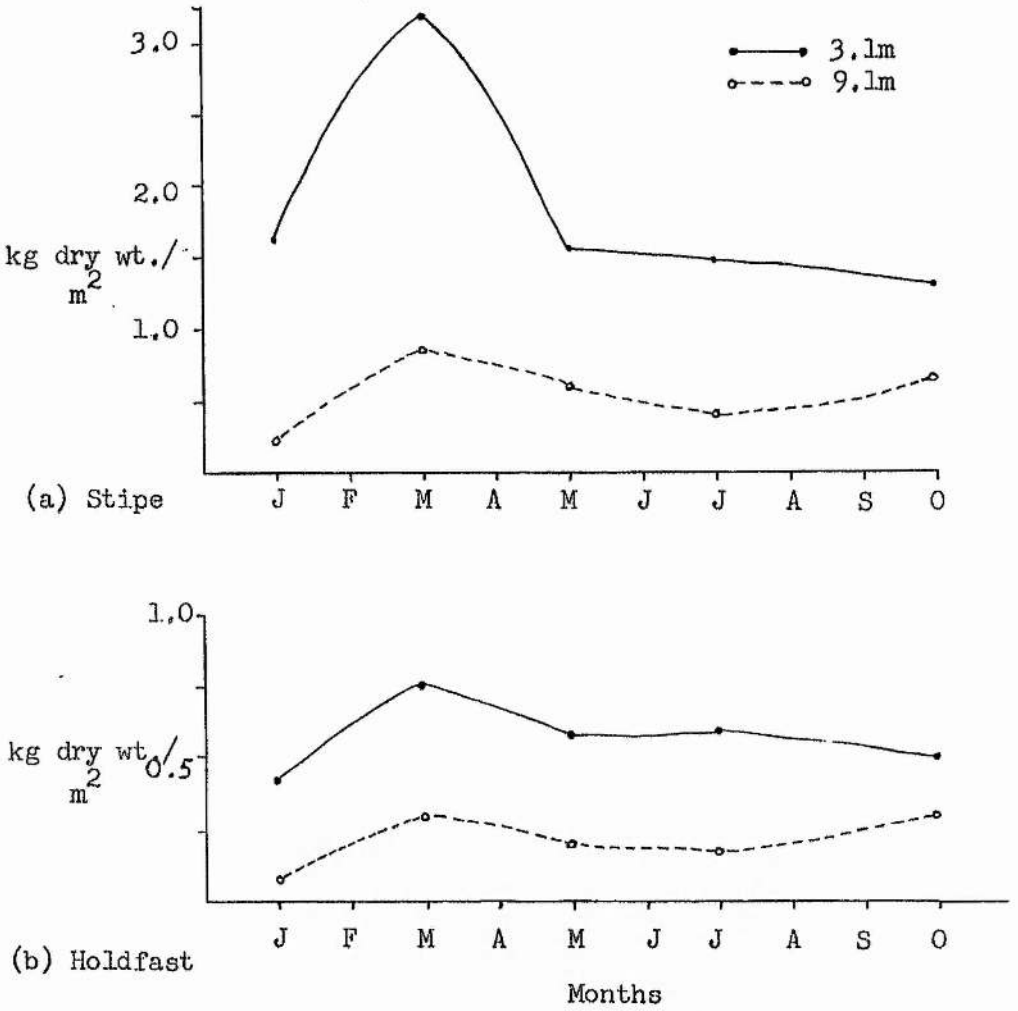


Figure 3:10 Seasonal variation in stipe and holdfast biomass at 3.1m and 9.1m.

The increment cropping technique is ideal for communities which show a marked annual fluctuation in biomass and where plants are subject to few

losses due to damage, grazing, reproduction etc. Where this is the case, and if the initial biomass at the start of the growing season is negligible, then the seasonal maximum biomass is a measure of the net annual productivity.

The mode of annual growth of the lamina of Laminaria hyperborea would appear to show that this tissue is particularly amenable to this form of analysis. Thus a new lamina is produced 'de novo' in January and grows rapidly, without marked fluctuation, to a seasonal maximum biomass in October. This represents the net production of lamina tissue and, if it can be assumed that little direct damage occurs to the lamina due to wave action, grazers, etc., then measurement of the seasonal maximum biomass is sufficient to give a value for the net annual lamina productivity. Losses due to respiration, exudation, sporogenesis etc., are irrelevant when a value for net production is required but for gross productivity all these losses have to be assessed and added to the net production.

The more serious criticism of the use of seasonal maximum lamina biomass as representing net productivity of the lamina is that, if damage to the lamina during the growing season was extensive, then the value for productivity would be an underestimate of the true value. In this study all the plants in a quadrat were used to estimate the net annual lamina production and no rejection of damaged individuals was made. The value obtained may therefore be an underestimate. Lüning (1970a) and Whittick (1969) have suggested lamina biomass is quite stable during the summer and that significant breakdown of the lamina occurs only after the maximum biomass is achieved.

The main damage to the lamina is likely to come from wave action, causing erosion of the lamina, and from grazers. Since wave action becomes progressively less severe during the time of rapid growth (from March/May to June/July) and the main grazer Patina pellucida L. is unlikely to reach the lamina until the later part of the season (see below) it can be assumed that the maximum lamina biomass recorded in October represents the net annual

lamina production. Graham & Fretter (1947) have shown that migration of Patina pellucida from the old lamina of L. hyperborea occurs in late autumn and very few limpets get onto the developing new lamina. It is likely that most of the individuals of Patina are lost with the old lamina when it is removed in May or June. Kain & Svendsen (1969) found that Patina may be present on the lamina from June to January, being most obvious in September and October and scarce in December and January.

For the perennial parts of the plant, the stipe and holdfast, the situation is more complex. Figure 3:10 shows that there is a seasonal variation in the biomass of these tissues but this was from a small number of samples each with a different age structure. Since the biomass of the stipe and holdfast is dependent upon age (Figure 3:3 and 3:5) then there is likely to be a large intrinsic variation in this biomass data regardless of seasonal differences. These graphs show trends in biomass changes in the stipe and holdfast but the seasonal maxima were not used to estimate net annual productivity.

The method used by John (1968) and Whittick (1969) was adopted to estimate the net annual primary productivity of the stipe and holdfast. This involves the use of simple age biomass relationships as used by Bellamy & Holland (1966) where 'growth curves' of Calluna vulgaris, obtained from increment croppings, were used to give a figure of net annual serial production. Kain (1971b) added the mean increments of stipe weights for each age group to the mean lamina weight for that age group and then added these values progressively from one age group to the next to give production data for various populations of L. hyperborea.

The mean individual plant stipe and holdfast dry weight for each age class at each depth has been plotted in Figures 3:3 and 3:5. This data was obtained for all the quadrats disregarding seasonal changes. The annual increment from one age class to the next (i.e. the slope of these graphs) indicates the net annual individual production that is likely to be achieved during that particular year. If the annual increment for each age class is

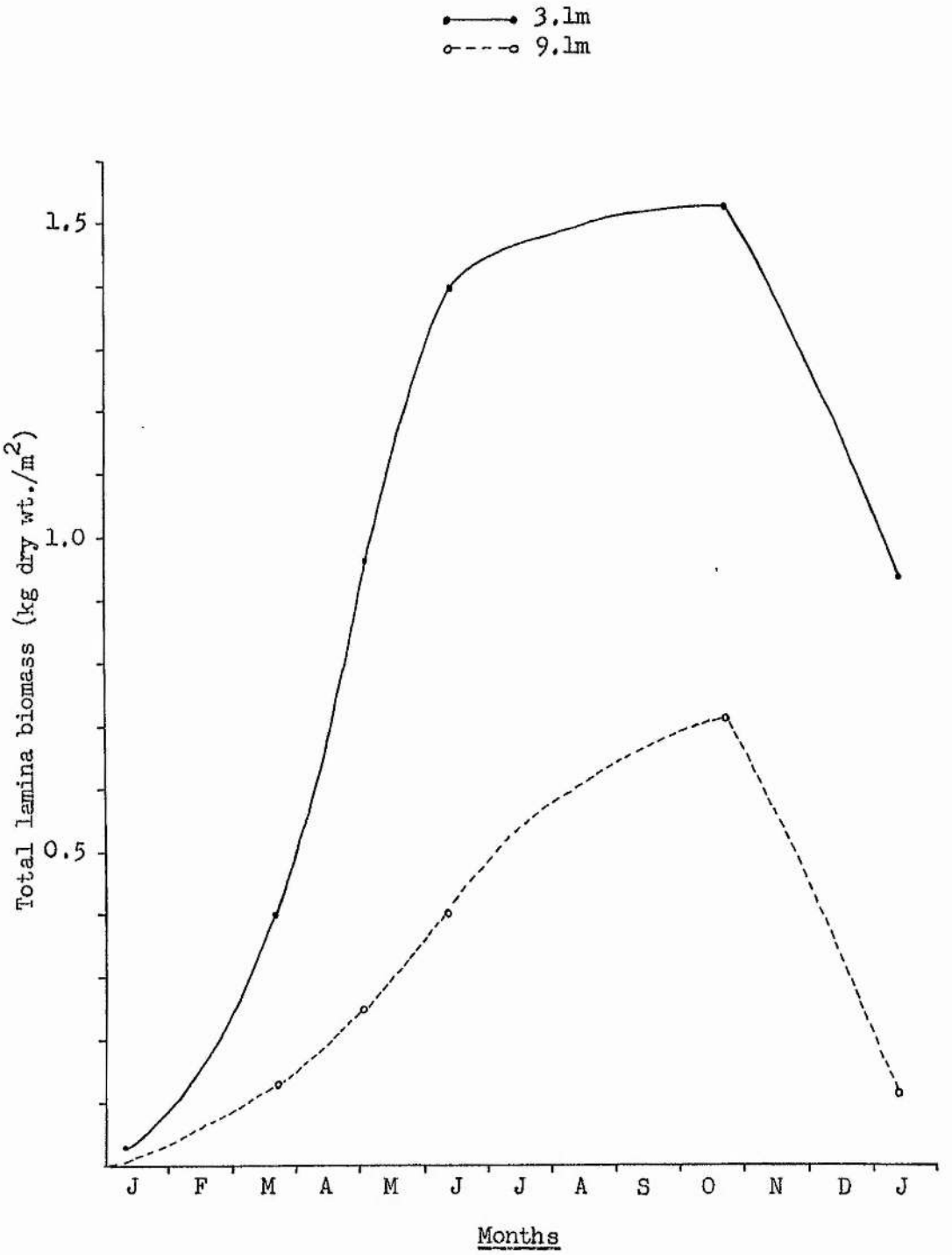


Figure 3:11. Seasonal variation in lamina biomass for the populations at 3.1m and 9.1m.

progressively totalled than a figure is obtained for the net annual production of biomass for that population. From a knowledge of the average numbers of plants in the area studied the net annual plot production can be estimated. Thus the annual increments for each age class are multiplied by the mean number of plants of that age found in a m^2 of sea-bed to give the net annual plot production (g/m^2) for that particular age class. The total net annual plot production is obtained by adding up all the values of net annual plot production for each age class. These values for stipe and holdfast, when added to the net annual plot production of the lamina (i.e. the seasonal maximum lamina biomass) give a figure for the net annual primary production in g dry weight/m^2 sea-bed/year.

Calculation of net annual primary productivity

The seasonal maximum lamina biomass at each depth was obtained from Figure 3:11 which shows that a maximum lamina biomass of 1.53 kg dry weight/m^2 sea-bed was found in October at 3.1m. The seasonal maximum lamina biomass of 0.71 kg dry weight/m^2 sea-bed at 9.1m was also found in October. These values represent the net annual production of these annual parts of the Laminaria plants.

The data for the stipe and holdfast tissues is set out in Tables 3:11 and 3:14. The first column for each tissue gives the average weight of tissue for that particular age group. The second column gives the annual increment of the tissue as the difference in biomass from one age group to the next. The third column gives the mean number of individuals/m^2 sea-bed in each age group. This data was obtained by considering all the quadrats at each depth. The annual increment of tissue in each age group was then multiplied by the mean number of individuals/m^2 of that age group to give the net annual plot production in g/m^2. Summation of the values of net annual plot production for each age group gave the total net annual plot production for the perennial parts of the plants. The data for stipe and holdfast tissue was then added to the annual lamina plot production to give a final figure for net annual primary plot production.

a) Net annual primary productivity at 3.1m.

Table 3:11

Net annual plot production of stipe and holdfast

Age (years)	Holdfast B	AI	N	NAPP	Stipe B	AI	N	NAPP
1	0.2±0.04	0.2	2	0.4	0.1±0.04	0.1	2	0.2
2	0.2±0.06	0	6	0 0,	0.2±0.04	0.1	6	0.6
3	0.8±0.3	0.6	6	3.6	0.8±0.1	0.6	6	3.6
4	1.7±0.3	0.9	7	6.3	2.8±0.5	2.0	7	14.0
5	7.2±1.7	5.5	7	38.5	17.7±4.0	14.9	7	104.3
6	11.0±2.5	3.8	3	11.4	32.6±8.3	14.9	3	44.7
7	36.7±3.1	25.7	3	77.1	112.9±14.6	80.3	3	240.9
8	38.5±3.5	1.8	4	7.2	121.6±7.8	8.7	4	34.8
9	39.2±7.2	0.7	2	1.4	124.6±6.2	3	2	6
10	42.9±6.5	3.7	2	7.4	109.7±10.5	-	2	-
			Total = 153.3				Total = 449.1	

where

B - mean plant biomass (g dry weight)

AI - annual increment

N - number of plants/m^2

NAPP - net annual plot production (g dry weight/m^2 sea-bed)

The annual plot production figures for the stipe and holdfast were added to the seasonal maximum lamina biomass to gived the total net annual primary productivity. The composite data are shown in Table 3:12.

Table 3:12
Net annual primary productivity (3.1m)*

Tissue	kg/m^2 sea-bed/year dry	organic a	metric tons/ha/year dry	organic b	calorific content of organic matter kcal/m^2 sea-bed/year c
Lamina	1.53	1.23	15.3	12.3	5841
Stipe	0.45	0.32	4.5	3.2	1461
Holdfast	0.15	0.10	1.5	1.0	458
Total	2.13	1.65	21.3	16.5	7760

*The following conversion factors were used:
a - From Table 3:1 organic content lamina = 80.2% dry weight
stipe = 71.5 "
holdfast= 66.8 "

b - 1 kg/m^2 = 0.1 metric ton/hectare

c - From Table 3:1, calorific content of lamina = 4.76 kcal/g organic matter
stipe = 4.55 "
Holdfast= 4.49 "

Assuming a growing season of 304 days from January to October and a conversion factor of x 0.47 for the carbon content of organic matter (Westlake, 1963), the net annual primary production figures in Table 3:12 can be converted to daily production figures as set out in Table 3:13.

Table 3:13
Growing season mean productivity (3.1m)

Tissue	g/m^2 sea-bed/year dry	organic	g/m^2 sea-bed/day dry	organic	gcarbon/m^2 sea-bed/day
Lamina	1530	1227	5.0	4.0	1.9
Stipe	449	321	1.5	1.0	0.5
Holdfast	153	102	0.5	0.3	0.2
Total	2132	1650	7.0	5.3	2.6

b) Net annual primary productivity at 9.1m

Table 3:14
Net annual plot production of stipe & holdfast

Age (years)	Holdfast B	AI	N	NAPP	Stipe B	AI	N	NAPP
1	0.2	0.2	1	0.2	0.2±0.05	0.2	1	0.2
2	0.4±0.06	0.2	2	0.4	0.5±0.2	0.3	2	0.6
3	1.0±0.2	0.6	1	0.6	0.9±0.2	0.4	1	0.4
4	2.9±0.9	1.9	2	3.8	2.6±0.6	1.7	2	3.4
5	11.8±2.9	8.9	3	26.7	21.2±5.2	18.6	3	55.8
6	15.8±2.5	4	3	12	41.2±6	20	3	60
7	17.5±2.8	1.7	3	5.1	45.6±6.9	4.4	3	13.2
8	22.5±5.7	5	2	10	55.5±9	9.9	2	19.8
9	23.3±5	0.8	1	0.8	66.3±8	10.8	1	10.8
10	26.1±4.7	2.8	1	2.8	79±22	12.7	1	12.7
			Total = 62.4				Total = 176.9	

The total net annual primary productivity for the 9.1m population is set out in Table 3:15.

Table 3:15

Net annual primary productivity (9.1m)

Tissue	kg/m^2 sea-bed/year dry	organic a	metric tons/ha/year dry	organic	calorific content of organic matter kcal/m^2 sea-bed/year b.
Lamina	0.71	0.62	7.1	6.2	3101
Stipe	0.18	0.13	1.8	1.3	579
Holdfast	0.06	0.05	0.6	0.5	213
Total	0.95	0.80	9.5	8.0	3893

a - From Table 3:1, organic content lamina = 87.9% dry weight
stipe = 75.2 "
Holdfast= 73.6 "

b - From Table 3:1, calorific content of lamina = 4.97 kcal/g organic matter
stipe = 4.35 "
Holdfast= 4.62 "

The data are converted to growing season mean productivities using the same factors as in Table 3:13 and are presented in Table 3:16.

Table 3:16.

Growing season mean productivity (9.1m)

Tissue	g/m^2 sea-bed/year dry	organic	g/m^2 sea-bed/day dry	organic	g carbon/m^2 sea-bed/day
Lamina	710	624	2.3	2.1	0.97
Stipe	177	133	0.6	0.4	0.21
Holdfast	62	46	0.2	0.15	0.07
Total	949	803	3.1	2.65	1.25

Net assimilation rate (NAR)

The net assimilation rate is the rate of dry weight increase at any instant on a leaf area basis (see chapter 10, Šesták, 1971). It can be calculated from the following formula:

$$NAR = \frac{W_2 - W_1}{t_2 - t_1} \cdot \frac{1}{LAI}$$

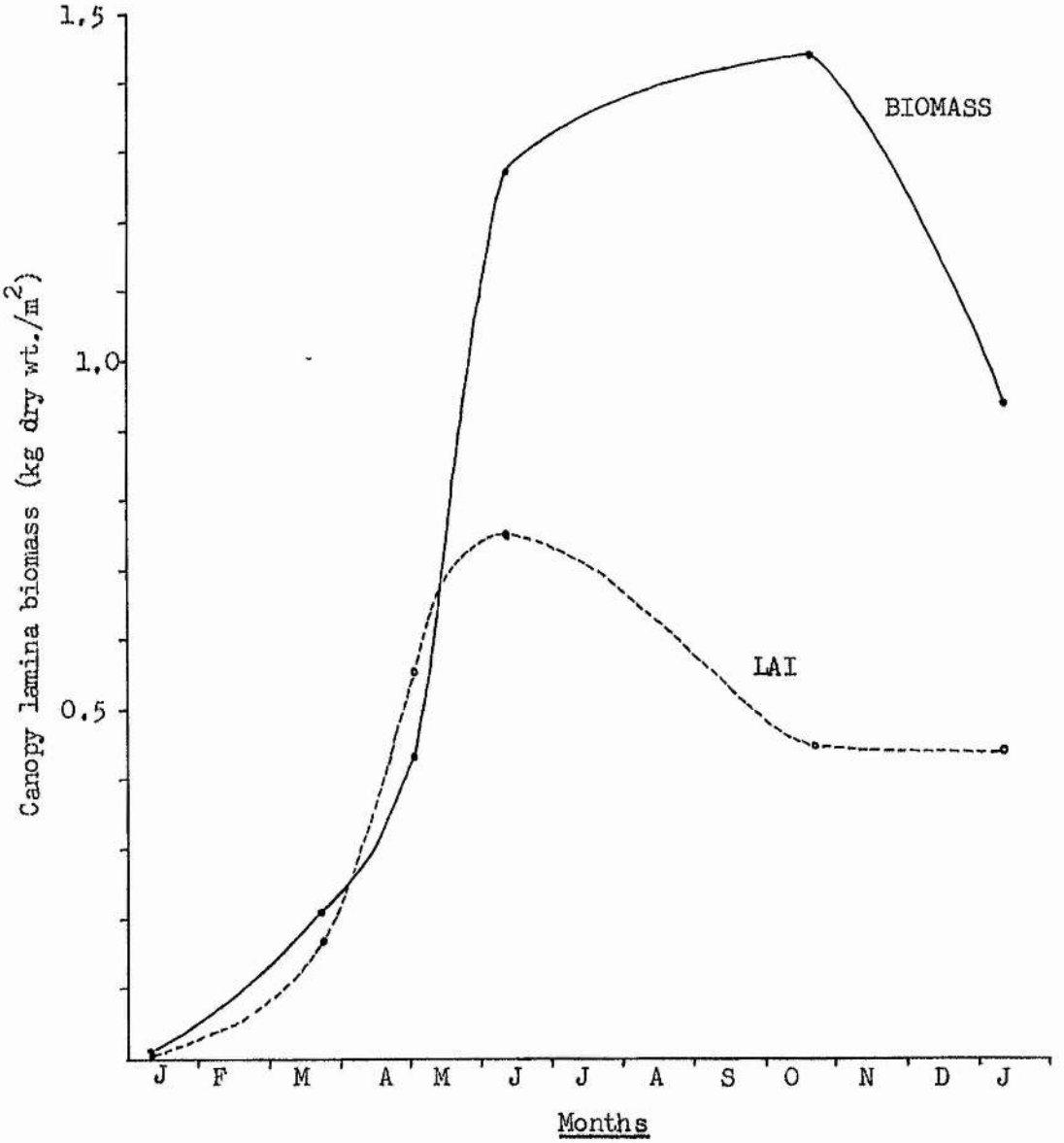


Figure 3:12 Seasonal variation in canopy lamina biomass and canopy LAI (3.1m)

where W_1 and W_2 are the lamina biomass at times t_1 and t_2 respectively, and LAI is the leaf area index. The mean values of W_1 and W_2 can be estimated from a smooth curve of the lamina biomass data at the beginning and end of each month. The mean LAI for each month has been calculated and a value for NAR at that particular time of year can thus be found. Shading experiments (e.g. Blackman & Black, 1959) have confirmed that a positive correlation exists between NAR and incoming radiation. The values obtained for NAR at various times of the year can be compared with the C^{14} estimates photosynthesis rates (see chapter 5).

The values for NAR have been calculated from the lamina biomass curve for canopy plants (7 to 10 years) shown in Figure 3:12. The data were restricted to the canopy forming plants so that a comparison could be made between the NAR from growth analysis and the estimates of production from C^{14} experiments carried out on these same older plants. Thus from this graph the values of lamina biomass at the beginning and end of the month under consideration were found (W_1 and W_2) and this biomass increment was converted into a production rate in terms of carbon accretion. Since the biomass increment was based on a unit area ($1m^2$) of sea-bed and the LAI for that particular month gave an estimate of the total lamina area present on the same unit area, then the LAI can be used to convert the production rate into the NAR. The LAI values from Table 3:9 are shown in Figure 3:12 and the median value of LAI (considered to occur in the middle of each month) has been used to convert production rate to NAR. The NAR has been calculated for all the months between January and August. An example is given of the calculation involved to obtain NAR from the production rate:

e.g. NAR in March

Lamina biomass increment (W_2-W_1)	= 250-130	g dry weight/m^2 sea-bed/ 31 days	
	= 120	"	
	= 3.87	g dry weight/m^2 sea-bed/day	
(Using factor of x 0.80 for 3.1m lamina (Table 3:1)	= 3.09	" matter "	
(Using factor x 0.47 Westlake, 1963)	= 1.46	g carbon/m^2 sea-bed/day	

This final figure represents the net daily productivity in March; it

is, however, based on a unit area of sea-bed, and the NAR is based on unit lamina area. Thus to convert this production value to NAR the value is divided by the mean value of canopy LAI for the month of March, which is 1.4.

NAR of new lamina in March = 1.46/1.4 g carbon/m^2 lamina/day
= 1.04 "

Other values of NAR for all the months of thegrowing season are given in Table 3;17.

Table 3:17

Mean monthly NAR for new lamina of canopy plants at 3.1m
(g carbon/m^2 lamina/day)

Month	January	February	March	April	May	June	July	August	September
NAR	7.63	2.15	1.04	0.61	1.23	0.32	0.08	0.07	0.06

The high values of NAR in January and February are noteworthy as it will be demonstrated in chapter 5 that there is no net photosynthesis occurring in the new lamina at this time. Growth analysis in this case gives a misleading impression that the tissue is assimilating carbon for growth; the high value is a reflection of the small area of lamina at this time (Figure 3:7), and the dry weight increment may be the result of translocation of compounds to the new lamina. Apart from this rather misleading result the NAR increases early in the growing season, reaching a peak in May and gradually falling off after this.

DISCUSSION

The mean biomass data found for the L. hyperborea forest at Arisaig indicate that biomass is reduced with depth.

The reduction in biomass over the depth range examined does not seem to adhere to the ecological hypothesis suggested by Walker (1947, 1958) but the standing crop estimates of Walker (1947 to 1958) are not strictly comparable to the estimates given here due to the different sampling techniques used. Walker (1958) correlated the decrease in biomass at depth with the decreased light conditions. However, it is clear from the data in Table 3:4 that the individual plant performance is often not markedly different in deeper water. This has also been noted by Walker (1948, 1950), Kain (1967, 1971b), and Lüning (1970a). John (1967) found that the individual performance of L. ochroleuca remained constant at all depths. Thus the low production at depth is explained in terms of a reduction in the density of individuals rather than individual performance. The physiological basis of the ability of L. hyperborea to grow well in deeper water has been investigated in later chapters. The reduction in density of plants in deeper water has been correlated with reduced light at the time of establishment and also with grazing pressure (Kain 1971a).

The biomass data presented show that this site is productive compared with data for other sites. Thus MacFarlane (1952) has compared the average biomass in Canada (Nova Scotia) of 6.5 to 32 kg fresh weight/m^2 with the average Scottish values of 5 to 8 kg fresh weight/m^2 (Walker, 1954c) and she has attributed the higher productivities in Canada to the greater tidal exchange in Nova Scotia waters. However, the maximum biomass of 36.7 kg fresh weight/m^2 recorded in the present study indicates higher values than the average value of 6 kg fresh weight/m^2 found by Walker (1954a) for Scottish waters. This is probably at least in part due to the sampling error of the spring grab used by Walker. However the biomass value may be compared directly with SCUBA collected samples. Thus Lüning (1969b) found standing crops of 11.1 kg fresh weight/m^2 at 2.0m and only 0.1 kg fresh weight/m^2

at 5.5m for a L. hyperborea forest in Helgoland. It would appear that the kelp forest at Arisaig is very much more productive than that in Helgoland. McFarland & Prescott (1959) found standing crops of between 4.4 and 5.8 kg fresh weight/m^2 for Macrocystis pyrifera. The organic weight of 4.7 kg/m^2 is high compared with the mean value of 2.1 kg organic matter/m^2 found for L. ochroleuca and S. polyschides (John, 1971).

The increase in plant longevity in more northern latitudes has been indicated by Kain (1967) as the main reason for the larger plants found in these latitudes. The forest studied at Arisaig would appear to be intermediate between the forests studied in Cornwall and Ireland (Larkum, 1972) and the Isle of Man (Kain, 1963), and the more northern Norwegian populations (Kain, 1967, 1971b).

The structure of the L. hyperborea forest indicates that shading effects and factors affecting the mortality of older plants are found. Thus the lack of intermediate lengths of stipes has been explained by Kain (1963) as due to the intense shading effect of the canopy.

The age distributions at the two depths indicated that a regular factor or factors act on the shallow population whilst the 9.1m population showed an irregular age distribution indicating that only some years are favourable for establishment. It seems that wave action, light and grazing affect establishment and the irregular distribution at depth reflects the occasional good year for establishment, whereas the regular factor of wave action may reduce the percentage of older plants seen at 3.1m (Kain, 1963).

The data for LAI indicate the high productivity of this species. Thus the maximum value recorded of 12.8 can be compared with the optimal LAI values of 3 to 6 for most arable crops and 6 to 11 for grass and fodder crops (Sěsták et al., 1971). Lüning (1969b) quotes values of 4.1 and 1.6 at 3.3m in a L. hyperborea forest in Helgoland, so the mean value of 3.4 at 9.1m in the Arisaig forest indicates that much better growing conditions are found there than at Helgoland.

The data for stipe growth at various ages show that the growth

of young plants at 3.1m may be reduced compared with the growth of similarly aged plants at 9.1m. Kain (1963) suggested that the dense forest canopy retards the growth of young plants by reducing the amount of light available to them. Experiments have been carried out 'in situ' to examine the effect of the canopy on the photosynthesis of the understorey plants (chapter 5).

There were no very small older plants at 3.1m so the 4 to 5 year plants which do not receive the better light afforded by the removal of older plants presumably die, although direct evidence of this was not found during diving operations. The small stipe biomass found for 10 year plants at 3.1m possibly represents the less well developed plants of a range of 10 year plants, the larger ones being selectively removed by grazing damage and wave-action, (Walker & Richardson, 1955; Kain, 1963; Kain & Svendsen, 1969).

Lamina morphology was seen to change with depth and position under the canopy. Thus the SLA of old plants at 3.1m was smaller than that of corresponding plants at 9.1m. Culture experiments described in chapter 8 indicate that light may be a controlling factor in this morphological change; Larkum (1972) has recently correlated it with wave action gradients which exist according to depth and position below the canopy.

The seasonal growth of the lamina of L. hyperborea has been investigated and it appears there are 3 phases in this growth; a 'lag' phase from January to March at 3.1m and from January to May at 9.1m and up to 9 g dry weight of canopy plant lamina may be produced by March in this growth phase. Whittick (1969) found that lamina biomass of 7 year plants at 1 to 2m was 5g dry weight by March. Lamina biomass was of the order of 2g dry weight for 2 to 5 year plants at 1m to 6m by March whilst at 12m all plants produced a lamina of less than 1g dry weight by March.

The lamina undergoes a rapid growth from March to June at 3.1m and from May to July or August at 9.1m. A slow growth phase occurs from June to the seasonal maximum biomass in October at 3.1m and from July to October at 9.1m.

Net annual primary productivities of 16.5 metric tons organic matter/hectare/year at 3.1m and 8 metric tons/hectare/year at 9.1m have been measured for the L. hyperborea forest studied. The growing season mean productivity was 2.6g carbon/m^2 sea-bed/day at 3.1m and 1.3gcarbon/m^2 sea-bed/day at 9.1m. Westlake (1963) gives numerous examples of productivity values for marine macrophytes and suggested the net annual organic productivity of sublittoral brown algae ranges from 10 to 31 metric tons/hectare/year. The value for the L. hyperborea forest studied is in the middle of this range. Laminaria longicuris was estimated to have an average value of 18 metric tons/hectare/year (Westlake, loc cit, from data of MacFarlane, 1952). John (1968) has calculated net annual productivities of a L. hyperborea forest in South East Scotland (55°55' N; 02° 09'W) of 21 metric tons organic matter/hectare/year at 3.0m and 9 metric tons/hectare/year at 7.6m. Lüning (1969a) found a maximum figure of the net annual productivity of the lamina only of L.hyperborea of 9.5 metric tons/hectare/year. John (1971) has estimated the net annual production of Laminaria ochroleuca and Saccorhiza polyschides in Spanish populations. The values for L. ochroleuca varied from 6 to 17 metric tons/hectare/year and the values for S. polyschides from 5 to 39 metric tons/hectare/year, the latter value being the highest figure yet reported for natural seaweed communities.

The value of the growing season mean productivity of 2.6g carbon/m^2 sea-bed/day at 3.1m compares favourably with data in Westlake's review; e.g. Laminaria longicuris with 3.9g carbon/m^2/day and Pinus sylvestris with 2.0g carbon/m^2/day. The value is within the range of highly productive ecosystems with 2.5 to 10g carbon/m^2/day (including coral reefs, evergreen forests, and intensive agriculture) quoted by Odum (1959).

CHAPTER 4

An 'In Situ' Growth Experiment.

Introduction

Experimental ecology in the sublittoral marine environment has involved the use of growth stations to investigate the effects of light, and temperature on the growth of macrophytic algae. These have either been set up 'in situ' using SCUBA (Neushul & Haxo, 1963; Neushul & Fowell, 1964; Lüning, 1969a, 1970a; John, 1968; H.T.Powell pers. comm) or have been set up from the surface (Sargent & Lantrip, 1952; Jones 1959; Sundene, 1962, 1964; Nicholson 1968).

Lüning (1969a) mounted plants of L. hyperborea on PVC plates and outgrowths of haptera fixed the plants to these plates which were attached to iron frames. The experimental treatments consisted of amputating the old lamina, amputating both the stipe and old lamina, and submerging these plants with controls in the same region the plants were taken from. Other plants were grown in complete darkness. The plants were brought to the surface on the frames at frequent intervals and the lamina areas measured. These experiments showed that plants grown in darkness could still produce a small new lamina. The isolated new laminae produced 'in situ' were the same size as the dark-grown plants, and amputation of the old lamina reduced the size of the new lamina produced compared to the control plant.

In a second series of experiments, Lüning (1970a) grew the similar four experimental groups in complete darkness, i.e. control; new lamina with old lamina but no stipe; new lamina with stipe but no old lamina; isolated new lamina. The results showed that the growth rates of the new lamina kept in darkness from February until May are considerably reduced if the old lamina is amputated but the new lamina was larger than the control when the stipe was removed.

Lüning (1969a; 1970a) concluded that the new lamina depends on reserve materials in the old lamina and that 'in situ' the stipe may provide

some reserves but in darkness the stipe exhibits a 'parasitic behaviour' and appears to act as a 'sink' for materials from the old and new lamina. Lüning also calculated that about 30% of the new lamina area produced during the period of rapid growth is due to reserve materials stored mainly in the old lamina. Lüning (1971b) has shown the existence of a translocation of C^{14} labelled assimilates from the old lamina to the developing base of the new lamina and, to a smaller extent, the stipe and holdfast in L. saccharina and L. hyperborea.

This chapter describes an 'in situ' growth experiment to investigate further the importance of the old lamina to the development of the new lamina and the effect of canopy shading on the subsequent development of the new lamina.

Experimental

A permanent quadrat was set up in February in the L. hyperborea forest at Fife Ness. The quadrat was made of $\frac{1}{2}$inch square section mild steel with inside length of 141cm and total area of 2 square metres. A central bar divided the quadrat into two areas, each of 1 m^2.

The quadrat was placed over a dense region of L. hyperborea on a rock pinnacle at 3.1m BMLWS. The quadrat was manoeuvred to encompass as many plant holdfasts as possible. In one half of the quadrat (1 m^2) the area was cleared of older, canopy forming plants, whilst the canopy was maintained in the other half.

The initial new lamina area was measured by placing the lamina flat on a formica board and tracing around the lamina edge with a pencil. The new lamina was very small then (0.1 to 14.8 cm^2) and could easily be drawn around. Each plant was tagged at the base of the stipe. There were a total of 9 plants in the cleared half of the quadrat; 2 of these had the old lamina amputated in February and 7 were left as controls. There were 10 plants analysed in the other half and 5 of these had their old lamina removed in February and the remaining 5 were left intact. The experiment was set up on 26.2.71 and all the tagged plants were removed from the quadrat on 20.8.71.

Each plant lamina was photographed and the lamina was cut up into small lengths which were laid flat in a polythene bag and photocopied; the lamina areas matched up on the photocopy and these were cut out and weighed. The lamina area was calculated from the weight of a known area of paper.

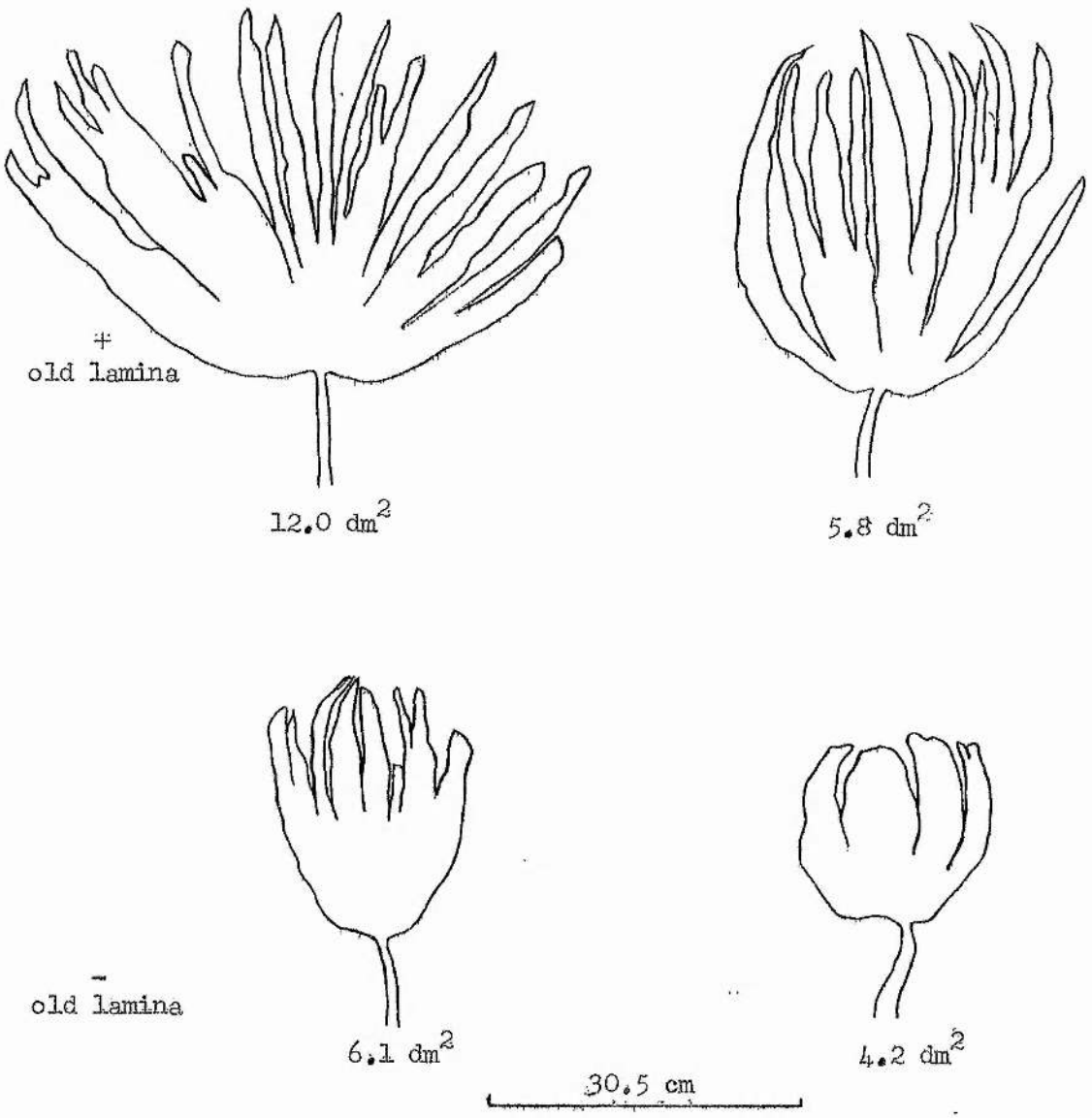


Figure 4:1 Effect of removal of the old lamina and the canopy on the growth of the new lamina.

Results

Table 4:1

The final lamina area of plants in the growth experiment

TREATMENT	CLEARED HALF ($1m^2$; 9 plants; 7-10L, 2-01.)	CANOPY HALF ($1m^2$; 10 plants; 5-16, 5-07.)
+ OLD LAMINA	12.0 ± 3.5	5.8 ± 2.3
− OLD LAMINA	6.1 ± 1.3	4.2 ± 0.7

(0.25) between + OLD LAMINA and − OLD LAMINA (cleared half)

(0.25) between + OLD LAMINA and − OLD LAMINA (canopy half)

(N.S.) between 12.0 ± 3.5 and 4.2 ± 0.7

(0.05) between 12.0 ± 3.5 and 6.1 ± 1.3

(N.S.) between 6.1 ± 1.3 and 5.8 ± 2.3

(0.25) between 12.0 ± 3.5 and 5.8 ± 2.3

1. Mean lamina areas are given in dm^2 ($100cm^2$) with SEM. The probability levels for 't' tests of differences between means is given in brackets between each treatment.
2. The average age of plants in the cleared half was 8 years, stipe length 40cm.
3. The average age of plants under the canopy was 7 years, stipe length 28cm.
4. Representative plants are shown in Figure 4:1 .

None of the differences are very significant due to the small number of plants in each treatment.* The poorest growth shown was that of plants under the canopy without the old lamina. The best growth was that of plants in the cleared half with the old lamina retained from February.

* Thus only the difference between final lamina area of plants in the cleared half with old lamina retained and plants under the canopy with the old lamina amputated was significant at the usually accepted 5% level of probability of t.

Discussion

This experiment confirms the findings of Lüning (loc cit), that the removal of the old lamina at the beginning of the growing season reduces the subsequent new lamina area given at the end of the season. This suggests that the new lamina relies on reserves which are translocated from the old lamina. Experiments to demonstrate this translocation are described in Chapter 9.

The rigorous shading effect of the canopy on the growth of understorey plants is indicated in this experiment. Thus the new lamina of control plants under the canopy is reduced compared to the control value in the cleared half, and, in fact, the new lamina produced under the canopy by control plants is the same area as the lamina of plants in the cleared half which had the old lamina amputated in February.

Amputated plants in the cleared and canopy halves had approximately the same lamina area. This suggests that the absence of the old lamina during the first few months of the year, when light conditions are frequently poor, has a critical effect on the subsequent lamina growth pattern. Thus, even when the amputated new lamina had as good light conditions as control plants in the cleared half, the photosynthesis of the new lamina could not make up for the initial absence of support from the old lamina. It is suggested that the supply of metabolites from the old lamina is critical for the establishment of an active meristem; the irretrievable loss had a compound interest effect and not even improved light could make up for the initial loss.

It must also be borne in mind, however, that the amputation of the old lamina may cause some direct, physical damage to the meristem and the results may be explained in terms of a wounding reaction. Nicholson (1968) could not eliminate a possible wounding effect as an explanation of data in field experiments after deblading plants of Nereocystis luetkana.

CHAPTER 5

The 'in situ' measurement of photosynthesis

Introduction

The importance of the measurement of the photosynthesis of marine algae under natural conditions must be stressed if any attempt is being made at analysing the effect of 'key' environmental factors on these rates and assessing the importance of photosynthesis during the growth of the plant. Without this data there is danger of a proliferation of hypotheses such as the probably undue emphasis placed on heterotrophy by Wilce (1967), as a means of supporting the growth of macrophytic algae under low light regimes in the Artic sublittoral. Jackson (1971) has reappraised Wilce's data and found little evidence to support the thesis that heterotrophy could support growth but found that exogenously supplied glucose may reduce the light requirement for photosynthesis. It was felt unnecessary to postulate heterotrophic supplement and Artic sublittoral plants, although slow growing, may survive purely by their own photosynthesis. Zaneveld (1966) found that 1.4% of incident light, i.e. a calculated 13 ft-c, penetrates a 2m thick ice cover to a depth of 20m and refuted the previous suggestion of Neushul (1963) that aphotic conditions exist under thick ice cover. The measurement of photosynthesis 'in situ' and long term production studies would be able to assess the importance of light and photosynthesis under these conditions.

Gail (1922) measured the photosynthesis of sublittoral red and brown algae 'in situ' and correlated the maximum amount of photosynthesis with the normal depth habitat of these algae; the maximum amount of photosynthesis by brown algae occurred at a depth of 1-8m whilst the maximum photosynthesis in red algae occurred at 10-25m. Printz (1939), Levring (1947, 1966, 1967), and Drew (1969) have carried out 'in situ' measurements of photosynthesis in several genera of marine algae in their studies of the zonation of sublittoral algae. Sargent & Lantrip (1952) showed, with the aid of 'in situ' measurements of photosynthesis, that translocation was necessary to supply organic compounds

to the growing tips of Macrocystis pyrifera, which were found to be below the compensation point. Tikhovskaya (1940) showed that photosynthesis of L.saccharina changed markedly with season and depth and that growth still took place during the polar night in December and January when no photosynthesis occurred. Blinks (1955) and Kanwisher (1966) report photosynthesis rates of littoral and sublittoral algae in various ecological situations. There have been extensive studies on the productivity of marine phytoplankton using 'in situ' techniques such as the Winkler oxygen method, and the C^{14} technique introduced by Steeman-Nielsen (1952). The information on primary productivity of marine phytoplankton has been reviewed by Strickland (1965).

A critique of the 'in situ' measurement of photosynthesis follows.

Advantages

1. The algal tissue is disturbed as little as possible from its natural environment. It is very likely that the removal of plants to the laboratory for measuring metabolism markedly affects the physiology of the plant. Kanwisher (1966) found that material kept in thelaboratory for some time before use showed decreased oxygen uptake even though the samples were not in the dark.
2. Photosynthesis of tissue under various ecological situations can be measured, such as at various depths and under the forest canopy.
3. It is difficult to simulate the complex marine environment in the laboratory. The many variables, such as light intensity and quality, and the almost continual flux of these variables, are difficult to reproduce and Strickland (1965) has reviewed the problems associated with incubators attempting to simulate underwater light.
4. A wide range of experiments can be carried out 'in situ' such as transplant studies, the effect of nutrient enrichment and pollutants on photosynthesis, and the method can be used for estimating productivity in algae that are not easily aged or are not suitable subjects for the increment cropping technique.

Disadvantages.

1. It is difficult to correlate changes in photosynthesis 'in situ' in anything more than a qualitative or semi-quantitative way with the more important

environmental variables, except perhaps the 'master' factor of light (Talling, 1961).

2. Where large tissue: volume ratios are used in studying photosynthesis of tissue discs the possibility of stagnation may occur. Whitford & Schumacher (1961) have emphasised the importance of fast currents around algal tissue which prevent a stratification of boundary layers.

3. The method is generally more inconvenient for obtaining adequate replicated measurements of photosynthesis than in the laboratory.

In the 'in situ' experiments no mechanical stirring of the medium in the incubation jars was carried out but the low tissue:volume ratio and the agitation of the jars on the buoyant platforms in currents meant it unlikely that much stagnation occurred in the jars.

Before presenting the data the interpretation of results from the C^{14} experiments is discussed and an example calculation set out.

Interpretation of 'in situ' C^{14} experiments

The C^{14} technique for measuring primary productivity, introduced by Steeman-Nielsen (1952), has been extensively used in marine productivity studies. It has much increased sensitivity over oxygen exchange techniques, especially in oligotrophic waters. At the same time an often acrimonious debate seems to have sprung up about the interpretation of results from C^{14} experiments, and in particular, disagreement has arisen as to whether uptake of C^{14} measures net photosynthesis, as favoured by Ryther (1954, 1956), or somewhere between net and gross fixation (Steeman-Nielsen & Hansen, 1959). Fogg (1963) has summarised the problems associated with the interpretation of C^{14} experiments and also pointed out the importance of exudation of organic compounds by marine algae.

If the C^{14} uptake is assumed to measure gross photosynthesis then the following criteria have to hold:

(i) that the C^{14} offered to the plant is in a readily utilisable form

(ii) that little loss of C^{14} through respiration occurs during the course of the experiment.

(iii) that little isotopic discrimination against C^{14} occurs

(iv) that little loss of Cl^4 occurs through exudation of labelled organic matter during the time course of an experiment.

These possible errors will be discussed in turn.

(i) The carbon source for photosynthesis

The pH range of seawater is relatively constant and rarely falls out of the range 7.8-8.3 (Skirrow, 1965), due to the buffer capacity of the carbonic acid system. At these pH values over 90% of inorganic carbon is in the form of the bicarbonate ion, HCO_3^-, in solution. There is little information available on the suitability of various carbon sources for photosynthesis in macrophytic algae but Raven (1968) has shown that a light stimulated electrogenic pump for HCO_3^- exists in Hydrodictyon africanum and Joliffe & Tregunna (1970) placed Laminaria as an 'Ulva' type in their experiments, in which pCO_2 and inorganic carbon (as bicarbonate) were varied and the effect on photosynthesis measured. The 'Ulva' types showed little change in the rate of photosynthesis when either pH or pCO_2 were varied but the rate was dependent on the concentration of inorganic carbon. It was concluded that these plants are able to use bicarbonate as their major source of carbon. The Cl^4 in the experiments described in this chapter was supplied as sodium bicarbonate -Cl^4.

(ii) Respiration losses during short term experiments.

In these experiments it is assumed that respiration is the same in the light as in the dark. Brown (1953) found that oxygen uptake by Chlorella was virtually unaffected by moderate light intensities. However it is now recognised that respiration and photosynthesis are unlikely to work independently. Hoch, Owens, and Kok, (1963), have indicated that photorespiration may replace dark respiration. In the face of lack of evidence for photorespiration in L. hyperborea it is assumed that respiration in the light is the same as that in the dark. (See section below).

Refixation experiment

An experiment was carried out to investigate whether light has any effect on the evolution of respiratory CO_2 from previous labelled substrates.

This experiment is similar to those of Steeman-Nielsen (1955).

Experimental

Rectangular lamina sections of 7.5cm^2 area were incubated in 28ml screw capped bottles with 1 µCi of sodium bicarbonate -Cl4 at 4.98 cal/cm^2/h and 7^{o}C for 2 hours. After this time the tissues were removed from the incubation medium, washed for 2 hours and transferred to 28ml bottles containing only Cl2 bicarbonate in the medium and kept either in the light or in the dark. At 1 hour periods for 4 hours the tissues were transferred to fresh medium and the water samples after each hour analysed for Cl4 content and this was assumed to represent the respiration of previously assimilated Cl4.

Results and discussion

The results are presented in Figure 5:1 below which shows the release of Cl_4 by the algal tissue in the light and dark. This experiment shows that during the normal time course of an 'in situ' experiment, i.e. about 4 hours, there is little loss of Cl_4 due to the respiration of previously fixed Cl_4, and further, refixation of this small amount of CO_2 respired is quite small

Thus,

(a) The value of Cl_4 respired as a percentage of initial counts fixed is very small: some 6.6% was respired in the dark bottle after 4 hours in the dark.

(b) refixation in strong light accounted for some 75% of the dark respiration of $14CO_2$: i.e. some 5% of initial Cl_4 fixed gets recycled.

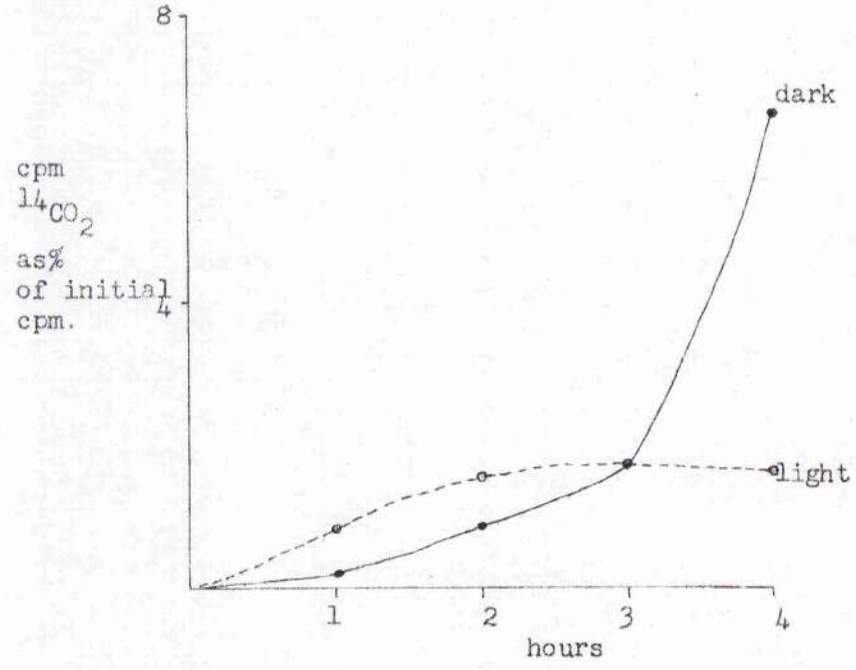


Figure 5:1 Loss of radioactive CO_2 in light and dark after assimilation of $NaHCO_3$-Cl_4

There is little loss of Cl^4 due to respiration in a 4 hour experiment and Drew (pers. comm.) found that when this experiment was carried out at different light intensities refixation was much reduced at lower light intensities than the strong light used above suggesting that, under the generally lower light levels found 'in situ' refixation would be negligible.

This experiment suggests that little Cl^4 labelled products reach the respiratory centres - the mitochondria - during the short time course of these experiments and of the small amount of Cl^4 respired, little is refixed. It is possible the labelled products are stored (Chapter 7). The uptake of Cl^4 by the alga during 'in situ' experiments is considered to represent gross photosynthesis as far as respiratory loss is considered. Steeman-Nielsen (1955) applied a 6% correction for respiratory losses and refixation in phytoplankton. If complete refixation of fixed Cl^4 occurred the uptake of Cl^4 would measure net photosynthesis (Ryther, 1954) but it appears from this data and that of Steeman-Nielsen (1955) that less than complete refixation occurs. It is possible that at the smaller diffusion gradients of carbon into the chloroplasts which may exist at lower light intensities, due to lowered photosynthesis rates, that much less refixation will occur.

Saunders (1964) considers that short term Cl^4 experiments measure gross photosynthesis and Rodhe (1958) feels it measures some value between gross and net. Fogg (1963) reports experiments on Anabaena where the Cl^4 method measured gross photosynthesis when compared with simultaneous measurements with the Winkler technique. An 'in situ' experiment was carried out in which Winkler and Cl^4 measurements of photosynthesis were made at the same time (see page 114) and this gave some further indication that Cl^4 uptake measures gross photosynthesis.

(iii) Isotopic discrimination

Where there is little or no evolution of CO_2 during irradiation, Yemm & Bidwell (1969) found that the discrimination against Cl^4 is only about 2%. As there appears to be little evolution of $^{14}CO_2$ during irradiation in Laminaria it would seem reasonable to assume no discrimination factor is necessary and none has been used.

(iv) Exudation losses

No experiments were carried out to investigate the loss of organic compounds by exudation in Laminaria. Sieburth (1969) has shown that considerable amounts of carbon may be exuded by macrophytic algae - up to 40% of the net carbon fixed in L. agardhii and L. digitata. However no correction was made for loss of C^{14} through exudation of labelled compounds and it is assumed that the major compound labelled during photosynthesis, mannitol (see chapter 7), is either stored in the vacuoles or redistributed in the plant. In any case, from the point of view of the growth of the Laminaria plant itself, exuded C^{14} labelled compounds make no contribution to growth although they may contribute to the ecosystem as a whole.

Example calculation of results from a C^{14} experiment

A specific example is given of the method of calculating primary productivity and photosynthetic efficiency from an 'in situ' experiment.

Location Arisaig ($56^{o}57'N$; $05^{o}52'W$) Date 24.3.70. Daylength 11.75h

(data from daily mean duration of possible sunshine at $56^{o}N$ in March; Meteorological Office)

Conditions Bright sun, calm sea. Temperature $7^{o}C$.

Details A 2 depth experiment at 4.6m and 10.1m with 3 discs of the old lamina of L. hyperborea at each depth. Time of incubation was 4 hours 50 minutes.

Integrator data (See Materials and Methods for further details)

Depth (m BMLWS)	Corrected Movement (mm/h)	% surface	cal/cm^2/h
Surface	12.4	-	15.5
4.6	4.4	35.7	5.5
10.1	0.5	3.8	0.6

The carbon 14 fixed by the 3 deep lamina sections (10.1m) is set out below to show how a figure for gross photosynthesis is derived. The data is corrected for dark fixation on a weight basis using a factor of 32.4 cpm/mg dry weight from a dark control jar and counts are converted to µgC using the specific activity of each water sample.

Sample	total cpm	mg dry weight	Total dark fixation	minus d.f.	Lamina area cm^2	cpm/cm^2 lamina/h	Specific activity of H_2O.	Gross Photo-synthesis (Pg) µgC/cm^2 lamina/h
1	199,500	129.2	4,186	195,314	24.7	1636	169	9.7
2	193,256	121.6	3,940	189,316	23.2	1688	169	10.0
3	316,548	160.1	5,187	311,361	28.1	2293	169	13.6

where Pg = gross photosynthesis.

Respiration data (see Addendum on page 206)

From the Respiration/Temperature graph on page 142 the interpolated lamina dark respiration rate (as carbon loss) at 7°C was 0.48 ugC/mg dry wt/h. The respiration for the total weight of tissue is found and this is then expressed on an area basis for comparison with the photosynthesis rate:

Sample	R_D (total dry wt). ugC/total mg/h	Area(cm^2)	Respiration (R_D) ugC/cm^2/h
1.	61.4	24.7	2.5
2.	57.8	23.2	2.5
3.	76.1	28.1	2.7

Net photosynthesis and carbon balance sheet

1. The gross photosynthesis value, P_G, is multiplied by the daylength for the particular latitude and time of year considered (data from Meteorological Office).
2. The respiration rate is multiplied by 24 to give the daily rate.
3. The difference between these values is regarded as the mean daily net photosynthesis in ugC/cm^2 lamina/day and is converted to gC/m^2 lamina/day.

Example

1. 9.7 x 11.75 - 113.98 ugC/cm^2/day
   2.5 x 24 - 60.00 "
   53.96 "

Thus the net photosynthesis for sample 1 is 0.54 gC/m^2 lamina/day

The data for each experiment is set out in the following way:-

Depth (m BMLWS)	Tissue	ugC/cm^2 lamina/h P_G	R_D	*P_N	P_G:R_D	R_D:P_G %	EDW (mg)	gC/m^2 lamina/day P_N
10.1	1.OI	9.7	2.5	7.2	3.9	25.8	129.2	0.54
	2. "	10.0	2.5	7.5	4.0	25.0	121.6	0.58
	3. "	13.6	2.7	10.9	5.0	19.9	160.1	0.95

where
P_G = gross photosynthesis
R_D = dark respiration
P_N = net photosynthesis; the value *P_N is $P_G - R_D$.
EDW = extracted dry weight in mg.

The ratio of gross photosynthesis to dark respiration is given (P_G:R_D)
The data for each 'in situ' experiment is the mean with its standard error (SEM).
The ratio of respiration to photosynthesis is given as a percentage.

Calculation of photosynthetic efficiency

The rates of photosynthesis can be converted into photosynthetic efficiency using the calorific content of the tissue from Table 3:1 , and the irradiance given in the integrator data. The photosynthetic efficiency is calculated from the ratio of energy fixed by the plant to the available radiant energy. It can be considered on the total incoming radiation or the fraction of the total which is utilisable. In this case the incoming radiation was measured at the restricted waveband of the green cinemoid filters in the integrators and corrected back to the total incoming radiation from 400nm to 700nm. It is assumed that only some 50% of the light measured by the meters is utilisable (Drew, pers. comm.; Ross & Nilson, 1965).

Example

Sample	$\mu gC/cm^2/h$ P_G	$cal/cm^2/h$			Photosynthetic efficiency (PE) (percentage utilisation)	
		P_G	total	50%	total	50%
1.	9.7	0.097	0.59	0.295	16.4	32.8
2.	10.0	0.100	"	"	16.9	33.9
3.	13.6	0.136	"	"	23.1	46.1

The conversions of gross photosynthesis to calorific content were as follows: From Table 3:1,

Average lamina calorific content = 4.9 kcal/g organic matter
Assume carbon content of organic matter = 47% (Westlake, 1963)

Therefore the calorific content of carbon = 10.4 kcal/g carbon
= 0.0104cal/μg carbon.

This factor was used to convert μg carbon fixed to calories. The efficiency of utilisation of solar energy obtained should, perhaps, not be viewed in absolute terms but regarded in comparative terms: the interest lies in the increased efficiency of utilisation of irradiance with depth. The maximum efficiency found in laboratory studies is thought to be 35%, assuming a quantum requirement of 8 (Daniels, 1956). Very high efficiencies in macrophytic algae have been noted before (Drew, 1969).

In this chapter various experiments, using an 'in situ' C^{14} technique, are described. These experiments were carried out to:-

(i) investigate photosynthetic capacity of various tissues of L. hyperborea and two other sublittoral algae, in the following ecological situations;

(a) at various depths

(b) both under and above the forest canopy

(ii) obtain data on the net carbon balance sheet and energy flow through the algal tissues at various times during the growing season.

(iii) see if any correlation could be made between the estimates of primary productivity from biomass studies and those from photosynthesis measurements.

(iv) investigate the importance of the light regime on photosynthesis 'in situ'

The experiments are described in four sections:-

Section 1 Photosynthesis of L. hyperborea at different depths and below the canopy.

Section 2 Experiments with other species of sublittoral algae.

Section 3 The effect of epibiota on the photosynthesis of L. hyperborea.

Section 4 Photosynthesis during the growing season.

1. Photosynthesis at different depths and below the canopy.

The biometric data in Chapter 3 indicated that, in some cases, the individual plant performance at 10.1m was significantly reduced compared to that at 3.1m, whilst, in other cases it was not. The effect of depth of submergence on the photosynthetic capacity of tissues of sublittoral macrophytic algae has been investigated. Similarly, the biomass age data and the 'in situ' growth experiment described in Chapter 4 indicated that the canopy layer exerts a considerable controlling effect on the growth of plants beneath it. Experiments are described to examine the effect of the canopy on the photosynthesis of tissues of algae.

Experiment A.

Location St. Andrew's Bay (56°20N;02°47'W) Date 6.11.69 Daylength 8.24h

Conditions Fairly bright;slight swell, falling tide Temperature 9°C

Details 2 depth experiment with 3 lamina discs (33.2 cm^2) of L.hyperborea at 3.1m and 9.1m.

Depth	$\mu gC/cm^2/h$ P_G	R_D	P_N	$P_G:R_D$	$R_D:P_G$	EDW	$gC/m^2/day$ P_N
3.1	4.9 ± 0.4	12.7 ± 1.6	7.7	0.39	256	821.8	-2.6 ± 0.4
9.1	4.3 ± 0.4	5.2 ± 1.1	0.9	0.83	121	342.8	-0.9 ± 0.3

Conclusions

The negative values for net photosynthesis at both depths indicate that growth is not occurring at this time of year which is in accordance with the biometric data. This may be due to reduced photosynthesis and increased respiratory 'load' since maximum lamina biomass is reached at this time of year. There is little change in photosynthesis with depth but the reduced respiratory loss at 9.1 is due to the smaller lamina weight per unit area found there (see EDW above).

Experiment B.

Location Arisaig (56°57'N; 05°52'W) Date 24.3.70 Daylength 11.75h

Conditions Bright sun, calm sea Temperature 7°C.

Details A 2 depth experiment at 4.6m and 10.1m with 3 discs of the old lamina of L. hyperborea.

Integrator data

Depth	Corrected Movement	% surface	cal/cm^2/h
Surface	12.4	-	15.5
4.6	4.4	35.7	5.5
10.1	0.5	3.8	0.6

Results

Depth	µgC/cm^2/h P_G	R_D	P_N	$P_G:R_D$	$R_D:P_G$ %	EDW	gC/m^2/day P_N
4.6	19.9 ± 2.2	2.8 ± 1.1	17.1	7.1	14.1	200.6	1.68 ± 0.5
10.1	11.1 ± 1.3	2.6 ± 0.7	8.5	4.3	23.4	137.0	0.69 ± 0.2

Since the new lamina was not used in this experiment the data for the old lamina was converted to that for the new lamina using an extrapolated factor from the average ratio of old lamina to new lamina photosynthesis during the spring growth period from other experiments: the ratio used was 1.4

(a) extrapolated new lamina net photosynthesis (4.6m) = $\frac{1.68}{1.4}$ = 1.2 gC/m^2/day

extrapolated new lamina net photosynthesis (10.1m) = $\frac{0.69}{1.4}$ = 0.49 "

(b) extrapolated new lamina gross photosynthesis (4.6m) = $\frac{19.9}{1.4}$ = 14.2 "

extrapolated new lamina gross photosynthesis (10.1m) = $\frac{11.1}{1.4}$ = 7.9 "

(c) extrapolated new lamina P:R ratio (4.6m) = $\frac{7.1}{1.4}$ = 5.1

extrapolated new lamina P:R ratio (10.1m) = $\frac{4.3}{1.4}$ = 3.1

Photosynthetic efficiencies

Depth	$\mu gC/cm^2/h$	cal/cm²/h			PE	
	P_G	P_G	total	50%	total	50%
4.6	19.9	0.199	5.54	2.77	3.6	7.2
10.1	11.1	0.111	0.59	0.295	18.8	37.6

Conclusions

The high value of the $P_G:R_D$ ratio and the positive value for net photosynthesis indicates growth is taking place at this time of year and that the old lamina is by no means a senescent organ at this time of year. It can be seen that there is an increase in the photosynthetic efficiency with depth. The slightly reduced carbon loss at 10.1m compared to that at 4.6m is a reflection of the 'thinner' lamina found at depth.

Experiment C.

Location Mousehole, Cornwall (50°07'N; 05° 32W) Date 17.7.70 Daylength 15.83h

Conditions Bright sun; calm sea, rising tide. Temperature 3.1m - 13°C
10.7m - 12.6°C
18.3m - 12.3°C

Details A 3 depth experiment using 3 lamina discs of L. hyperborea at 3.1m, 10.7m, and 18.3m.

Integrator details

Depth	Corrected movement	% surface	cal/cm^2/h
surface	14.6	-	18.3
3.1	10.2	69.9	12.8
10.7	2.8	19.2	3.5
18.3	1.3	8.8	1.6

Results

Depth	µgC/cm^2/h			$P_G:R_D$	$R_D:P_G$	EDW	gC/m^2/day
	P_G	R_D	P_N				P_N
3.1	8.3	3.4	4.9	2.4	41	259.2	0.5
10.7	5.9	3.9	2.0	1.5	66	288.1	-0.01
18.3	5.4	1.5	3.9	3.6	27.8	106.6	0.5

Photosynthetic efficiencies

µgC/cm^2/h		cal/cm^2/h			PE	
Depth	P_G	P_G	total	50%	total	50%
3.1	8.3	0.083	12.8	6.4	0.7	1.3
10.7	5.9	0.059	3.5	1.75	1.7	3.4
18.3	5.4	0.054	1.6	0.8	3.4	6.8

Conclusions

Again the photosynthetic efficiency was found to increase with depth. The ratio of gross photosynthesis to respiration was low compared to the data

in experiment B. It should also be noted that at 10.7m the net photosynthesis was negative indicating the reduced growth at this time of year. Figure 5:2 below shows the gross photosynthesis values plotted against depth. The values of respiration rate and the irradiance (percentage of corrected surface integrator response) for each depth are also shown. It can be seen from this graph that photosynthesis is not reduced to the same extent as irradiance indicating the increased photosynthetic efficiency at depth. The respiration rate increase to a maximum value at 10.7m and then decreases. This reflects the lamina biomass changes seen in the extracted dry weight data for the lamina discs at each depth.

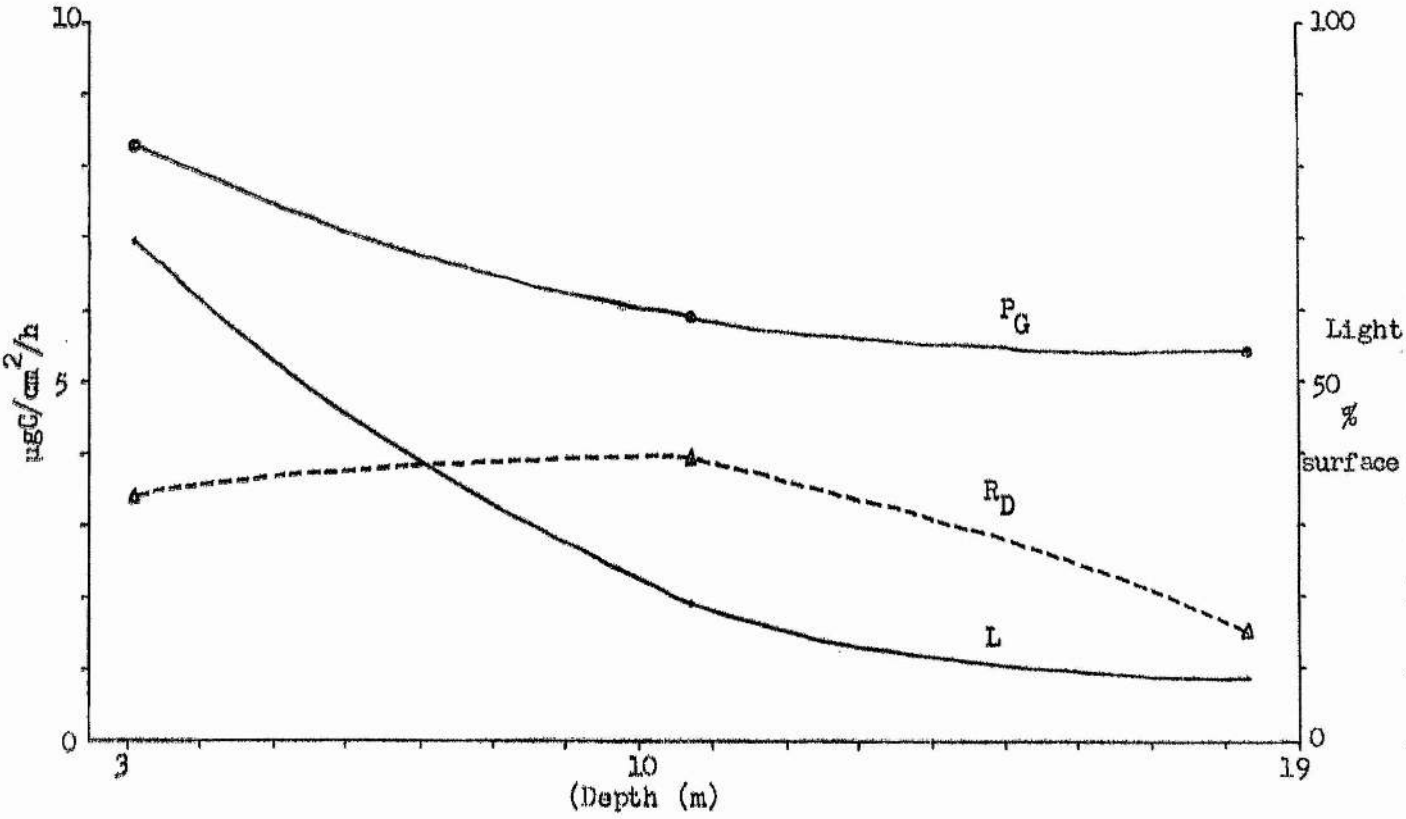


Figure 5:2 Photosynthesis (P_G), respiration (R_D) and light intensity (L) at various depths.

Experiment D.

Location. Mouschole, Cornwall. Date 8.7.70 Daylength 15.83h.

Conditions. Dull, heavy rain; slight swell increasing to a 6' swell at the end of the experiment. Temperature 3.1m - 13.6°
10.7m - 13.2°
18.3m - 13.0°

Details. A 3 depth experiment using 3 lamina discs of L. hyperborea. The 3.1m platform had worked off its position by the end of the experiment and the jars were shaded so the results at this depth maybe somewhat low.

Integrator data.

Depth	Corrected movement	% surface	cal/cm^2/h
surface	3.9	-	4.9
3.1	2.7	68.8	3.4
10.7	0.9	23.6	1.2
18.3	0.5	11.9	0.6

Results.

Depth	µgC/cm^2/h			P_G:R_D	R_D:P_G	EDW	gC/m^2/day
	P_G	R_D	P_N				P_N
3.1	8.2±0.45	1.6±0.2	6.6	5.1	19.5	141.2	0.91±0.11
10.7	7.0±0.52	2.7±0.3	4.3	2.6	38.6	194.8	0.47±0.15
18.3	3.9±0.32	1.3±0.03	2.6	3.0	33.3	93.6	0.31±0.05

Photosynthetic efficiencies

µgC/cm^2/h		cal/cm^2/h			PE	
Depth	P_G	P_G	total	50%	total	50%
3.1	8.2	0.082	3.4	1.7	2.4	4.8
10.7	7.0	0.070	1.2	0.6	5.8	11.7
18.3	3.9	0.039	0.6	0.3	6.5	13.0

Conclusions

The irradiance levels recorded in this experiment are much lower than those in the previous experiment (C) and it can be seen that the efficiency of of the utilisation of light at each depth has increased with reduced light intensities.

Experiment E.

Location Mousehole Date 12.7.70 Daylength 15.83h

Conditions Bright Sun; calm sea. Temperature 4.6m - 13.0°
18.3m - 12.2°

Details A 2 depth experiment with lamina, stipe, and holdfast tissues of L. hyperborea. The samples at 4.6m were placed under the canopy and the samples at 18.3m were left in the open, unshaded community found at this depth. Plants used at 4.6m were 4 years old and selected from below the canopy: plants at 18.3m were 2-3 years old.

Integrator details

Depth	Corrected Movement	% surface	cal./cm^2/h
surface	13.6	-	17.0
*4.6 (below canopy)	-	-	-
18.3 (open)	1.23	9.0	1.1

*The irradiance below the canopy was below the sensitivity of the integrator, i.e. about 0.4 cal/cm^2/h.

Results

Location	Depth	Tissue	µgC/cm^2/h			$P_G:R_D$	$R_D:P_G$	EDW	gC/m^2/day
			P_G	R_D	P_N				P_N
Below canopy	4.6	lam.	1.2±0.1	2.9±0.14	-1.7	0.4	242	214.6	-0.49
		stipe	0.1	1.3	-1.2	0.1	1300	278.6	-0.30
Open	18.3	lam.	6.8±0.1	2.4	4.4	2.8	35	168	0.51
		stipe	1.1	0.9	0.2	1.2	82	191.3	-0.04

The holdfast tissue showed some fixation of C14 but the data could not be expressed on an area basis. The data is given on a weight basis and compared with stipe data.

	µgC/mg dry weight/h	
Location	Holdfast	Stipe
Below canopy (4.6m)	0.02	0.06
Open (18.3m)	0.05	0.22

Figure 5:3 shows the gross photosynthesis for lamina and stipe tissue at the two depths with the above canopy lamina data from 3.1m in experiment C.

Conclusions The main conclusions from this experiment are:-

(1) The photosynthesis of the lamina, stipe, and holdfast tissues is markedly reduced below the canopy at 4.6m compared to the photosynthesis at 18.3m.

(2) The tissues are below the light compensation point for photosynthesis under the canopy, whilst at 18.3m only the lamina shows a net uptake of carbon.

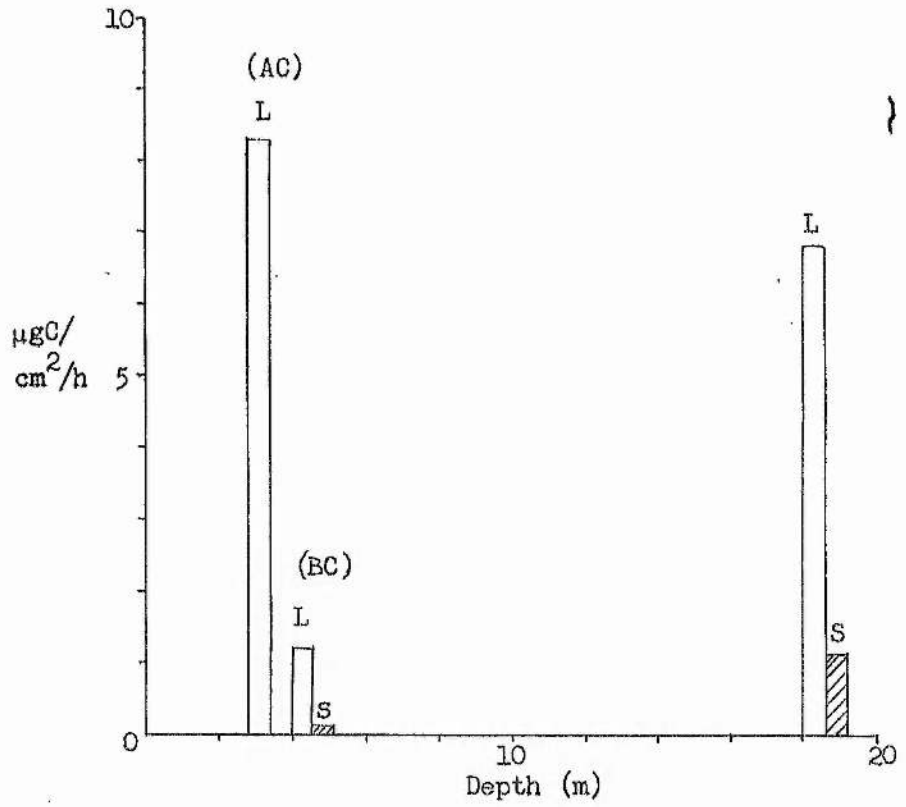


Figure 5:3 Gross photosynthesis of lamina (L) and stipe (S) above (AC) and below (BC) the canopy at 3-4m and at 18.3m

2. Experiments with other species of sublittoral algae.

In this section the photosynthesis of two other sublittoral algae has been investigated. This data allows comparison of the productivity of the main members of the kelp forest ecosystem and reveals some information on the competitive performance of these species. Kain (1969) found that light probably does not act as an ecologically differentiating factor on the growth of the gametophytes and early sporophytes of L. hyperborea, L. digitata, L. saccharina, Saccorhiza polyschides but that temperature sometimes may. The effects of light on the photosynthesis of the more mature stages of the sporophytes of these competitor species has not been investigated in great detail to date.

(a) Laminaria saccharina

Experiment F.

Location Lismore Island, Oban 56°25'N; 05°28'W) Date 20.6.69 Daylength 17.41h

Conditions Dull, overcast, rain; sea calm until 16.30h when squall blew up.

Temperature Depth	°C
3.1	9.6
12.2	9.9
21.3	10.0

Details A 3 depth experiment using 3 lamina discs (33.2cm^2) plus a dark fixation control at each depth. No irradiance data was obtained in these experiments, and the dark respiration value used was that from the RT curve for L. hyperborea. That there is probably not much variation in respiratory rates between members of the Laminariacae is seen in the comparative data set out in Table 5:1 .

Table 5:1

Comparative data on respiration in Laminaria spp.

Species	°C	Q_{O_2} µl O_2/mg dry wt/h.	Author
L.digitata	17	0.12	Spector, 1956
L. digitata	17.4	0.72	Milthorpe, 1949
L. digitata	19.0	0.64	Shaw, 1960.
L. hyperborea	18.0	0.63	this thesis
Laminaria sp.	20.0	0.20	Kanwisher, 1966.

Results

Depth	$\mu gC/cm^2/h$			$P_G:R_D$	$R_D:P_G$	$gC/m^2/day$
	P_G	R_D	P_N			P_N
3.1	11.3	1.3 ± 0.03	10.0	8.7	11.5	1.7
12.2	5.8 ± 0.1	2.0 ± 0.2	3.8	2.9	34.5	0.5
21.3	0.9 ± 0.2	2.4 ± 0.5	-1.5	0.4	266.7	-0.4 ± 0.2

Conclusions

The photosynthesis rates from this experiment can be compared with the data in experiment O, page113, which was carried out at the same time of year. In general the rates compare favourably with other sublittoral species. The growth of Laminaria saccharina is still rapid at this time of year (Parke, 1948b), as indicated in the high photosynthesis to respiration ratio. The finding of Tikhovskaya (1940) that photosynthesis in June is below compensation point at 10m is confirmed in the data for net production at 21.3m. However, conditions were poor during this experiment and net production was found at 21.3m in the next experiment run under better conditions.

Experiment G.

Location Lismore Island Date 21.6.69 Daylength 17.41h

Conditions Bright Sunny spells; Calm Sea Temperature 3.1m - 9.6°
12.2m - 9.9°
21.3m - 10.0

Details A 3 depth experiment using 3 lamina discs of L. saccharina at each depth.

Results

Depth	$\mu gC/cm^2/h$			$P_G:R_D$	$R_D:P_G$	$gC/m^2/day$
	P_G	R_D	P_N			P_N
3.1	15.7 ± 0.72	1.9 ± 0.21	13.8	8.3	12.1	2.3 ± 0.11
12.2	6.9 ± 0.68	2.1 ± 0.42	4.8	3.3	30.4	0.6 ± 0.22
21.3	5.2 ± 0.51	2.3 ± 0.22	2.9	2.3	44.2	0.39 ± 0.15

Conclusions

This experiment shows that in good conditions reasonable net fixation by tissues of L. saccharina is possible at substantial depth.

(b) Saccorhiza polyschides

Norton & Burrows (1969a) showed that this species is a fast growing annual and may be considered an opportunist alga capable of colonising any space that becomes available in the sublittoral region. It is of interest thus to examine the photosynthetic capacity of this plant compared with that of the more slower growing laminarians.

Experiment H.

Location Mousehole Date 10.7.70 Daylength 15.83h

Conditions Bright with drizzles; calm sea, slight swell. Temperatures Depth °C

3.1m - 13.1
10.7m - 12.3
18.3m - 12.2

Details A 3 depth experiment using 3 lamina discs (33.2cm^2) of S. polyschides. The 3.1m lamina discs were very thick and had some epizoic bryozoans growing on them. These surfaces were kept on the underside during incubation (see also experiment J).

Integrator data

Depth	Corrected Movement	% surface	cal/cm^2/h
surface	6.8	-	8.5
3.1	4.1	60.3	5.1
10.7	1.7	25.0	2.1
18.3	0.7	10.0	0.9

Results

Depth	µgC/cm^2/h			$P_G:R_D$	$R_D:P_G$	EDW	gC/m^2/day
	P_G	R_D	P_N				P_N
3.1	23.9	8.1	15.8	2.9	33.9	618.9	1.84
10.7	12.3±0.39	1.5±0.12	10.8	8.2	12.2	110.8	1.57±0.08
18.3	8.5±1.4	1.3±0.12	7.2	6.5	15.3	105.0	1.03±0.25

Photosynthetic efficiencies

Depth	$\mu gC/cm^2/h$ P_G	$cal/cm^2/h$			PE	
		P_G	total light	50% light	total	50%
3.1	23.9	0.24	5.1	2.55	4.7	9.4
10.7	12.3	0.12	2.1	1.05	5.7	11.4
18.3	8.5	0.09	0.9	0.45	10.0	20.0

Conclusions

This experiment included the highest gross photosynthesis recorded - at 23.9 $\mu gC/cm^2$ lamina/h - and indicates the high metabolic rate of this alga. This is obviously of great advantage as the species is annual and has to establish itself rapidly in gaps in the L. hyperborea forest. Photosynthetic efficiency increased with depth and a reduction in lamina weight per unit area was found in deeper samples.

Experiment I.

Location Mousehole Date 15.7.70 Daylength 15.83h.

Conditions Intermittent sun; moderate swell Temperatures Depth °C

3.1m - 12.5

19.8m - 11.6

Details A 2 depth experiment with lamina, stipe, and bulb tissue of S. polyschides above and below the L. hyperborea canopy at 3.1m, and in the open community at 19.8m.

Integrator data.

Depth	Corrected movement	% surface	cal/cm^2/h
surface	10.3	-	12.9
3.1m above * canopy	-	-	-
below canopy	-	-	-
19.8m open	0.52	5.0	0.7

*the integrator above the canopy did not work.

Results

Depth	Tissue	µgC/cm^2/h			$P_G:R_D$	$R_D:P_G$	EDW	gC/m^2/day
		P_G	R_D	P_N				P_N
3.1m above canopy	L	23.3	1.5	21.8	15.5	6.4	113.2	3.33
	S	12.2	1.7	10.5	7.2	13.9	358.3	1.52
3.1m below canopy	L	1.8	2.2	-0.4	0.8	122	164.8	-0.24
	S	-	-	-	-	-	465.8	-
19.8m	L	5.8±0.25	1.8±0.15	4.0	3.2	31	122.5	0.49±0.08
	S	2.8	1.2	1.6	2.3	42.9	255.9	0.16

Some fixation had occurred by the bulb tissue and is expressed on a weight basis.

Location		µgC/mg dry weight/h Bulb	Stipe
above canopy	3.1m	0.32	1.07
below canopy		0.23	0.07
Open	19.8m	0.24	0.40

Conclusions

The highest net photosynthesis recorded in these experiments was of lamina tissue of S. polyschides above the canopy at 3.1m.

The canopy of the L. hyperborea has a large shading effect on tissues of S. polyschides and all tissues here were below compensation. Thus the findings of Norton & Burrows (1969a) that the shade of the canopy may be an important factor inhibiting the development of S. polyschides and thus limiting the upward extension of the Saccorhiza zone, are substantiated in this experiment.

3. The effect of epibiota on the photosynthesis of L. hyperborea.

Kitching (1941) described the dense epiphytic populations of Rhodophyceae on the stipes and holdfasts of L. hyperborea. Marshall (1960) found 24 species of algae as epiphytes and the four major species found were Rhodymenia palmata, Phycodrys rubens, Membranoptera alata, and Ptilota plumosa. Whittick (1969) examined the productivity of the epiphytes of L. hyperborea and suggested that the stipe, with its dense covering of epiphytes, "must be mainly dependent on assimilates translocated from the lamina".

Clendenning & Sargent (1958) investigated the effect of the epizoic populations growing on Macrocystis pyrifera and, in particular examined the effect of heavy encrustations of Membranipora membranacea on the photosynthetic capacity of the lamina tissue.

As little is known of the effect of these epibiota on the photosynthetic capacity of L. hyperborea 'in situ', an experiment was carried out to investigate this effect.

Experiment J.

Location Mousehole Date 18.7.70 Daylength 15.83h

Conditions Cloudy, dull with some bright periods; calm sea, slight swell.

Temperature 3.1m - 13°

Details A one depth experiment at 3.1m, with 2 replicates of each of the following treatments:-

Above canopy	Below canopy
Stipe + epiphytes	Stipe + epiphytes (red algae)
Stipe - epiphytes	Stipe - epiphytes
Lamina + epizoans	Lamina + epizoans
Lamina - epizoans	Lamina - epizoans (Membranipora)

Heavily encrusted lamina and stipe sections were selected and (-) treatments were carefully scraped clean of epiphytes taking care not to damage the Laminaria tissue, or clean areas of lamina were selected.

Results

Location	treatment	$\mu gC/cm^2/h$ P_G	R_D	P_N	$P_G:R_D$	EDW	$gC/m^2/day$ P_N
Above canopy	S +	4.2±0.8	10.3±2.0	-6.1	0.4	3050	-1.81
	S -	3.4±1.8	2.8±1.0	0.6	1.2	596.2	-0.13
	L +	9.6±2.4	7.3±0.4	2.3	1.3	548.2	-0.23
	L -	9.6±0.4	3.3	6.3	2.9	243.6	0.73
Below canopy	S +	0.4	9.8±0.5	-5.1	0.04	3063.6	-2.3
	S -	-	-	-	-	608.5	-
	L +	0.14±0.05	6.4±0.5	-3.3	0.02	485.6	-1.5
	L -	0.45±0.06	4.1±0.1	-2.3	0.11	307.5	-0.9

The respiration (R_D) in the case of stipe plus epiphyte treatments was worked out using the stipe weight alone, but the lamina plus epizoan (or 'encrusted kelp complex', Clendenning & Sargent, 1958) respiration was worked out on the combined lamina plus epizoan weight. To see if the additional weight of epizoan affects the total respiratory complement greatly the weight of the epiphyte-free lamina was used in the L (+) treatments.

(a) In the case of the L (+) treatment above the canopy the gross photosynthesis is the same as for the L(-) treatment so the use of the L(-) weight gives a positive value for P_N of 0.73 gC/m^2 lamina/day. Thus the presence of the epizoan makes no difference to either the gross or net fixation of the lamina tissue beneath it in reasonable light conditions.

(b) Below thecanopy the gross photosynthesis is somewhat reduced in the L(+) treatment and when the L(-) weight is used to correct for respiratory loss the net photosynthesis given is -0.96 gC/m^2/day, which, although it is still negative, is somewhat reduced compared to the value obtained when using the weight of lamina plus epizoan for the respiratory correction.

Fixation of Rhodophyceae.

Although the fixation by the epiphytic red algae on the stipe

sections could not be satisfactorily expressed on an area basis some indication of the amount of fixation can be obtained on a weight basis. Data for Lamina (-) of L. hyperborea is given for comparison in Table 5:2.

Table 5:2

Location	Fixation by epiphytes µgC/mg dry weight/h	
	Epiphytes	L. hyperborea (-)
Above canopy	1.13	1.53
Below canopy	0.03	0.23

Conclusions

The main conclusion from this experiment is that the encrusting bryozoan colonies of Membranipora membranacea and the epiphytic Rhodophyceae (mainly Rhodymenia palmata, Phycodrys rubens, and Delesseria sanguinea) have little effect on the gross photosynthesis of the lamina and stipe of L. hyperborea. Thus, above the canopy the values of gross photosynthesis were not significantly different in the presence of epibiota, and the effect of Membranipora was to increase the respiratory loss of the encrusted kelp complex but had no effect on the net photosynthesis of the lamina tissue beneath it. This result is similar to that of Clendenning & Sargent (loc cit), who found no inhibition of photosynthesis encrusted Macrocystis blades in saturating light intensity.

Membranipora does however, reduce the gross photosynthesis of lamina tissue below the canopy and this is in accord with the findings of Clendenning & Sargent (loc cit) who indicated that the compensation point of encrusted blades of Macrocystis was raised compared to clean kelp and that up to 50% higher light intensities were required for photosynthesis and growth under the bryozoan colonies at limiting light intensities.

The fixation of the epiphytes indicated quite a high value above the canopy but a low value below the canopy compared to L. hyperborea. This is somewhat surprising considering the general 'shade' preference of these algae. It is possible that the position of incubation on the bed-rock was below the compensation of these algae or the epiphyte population may have been made up largely of less shade-loving species such as Phycodrys (Marshall, 1960).

4. Photosynthesis during the growing season

In order to examine the interrelationships between photosynthesis and respiration of tissues of L. hyperborea during the growing season several 'in situ' C^{14} experiments were carried out at different times from February to July. In this way a carbon balance sheet could be obtained and an assessment made of the importance of light and temperature on growth. Experiments were carried out in February, March, April, May, June and July with lamina, stipe, and holdfast tissue incubated above and below the forest canopy.

Experiment K February

Location Fife Ness (56°17'N: 02°36'W) Date 16.2.71. Daylength 9.48h.

Conditions Dull; heavy swell, very poor visibility underwater Temperature 5.2m 5°C

Details A one depth experiment with lamina, stipe, and holdfast tissue incubated above and below the forest canopy. Due to the breakage of injector needles C^{14}-bicarbonate was injected into some jars at the surface. This was done speedily and significant exposure to light was unlikely as surface light was low.

Integrator data.

Location		Corrected movement	% surface	cal/cm^2/h
5.2m	Surface	2.7	-	3.4
	Above canopy	0.7	26	0.87
	Below canopy	0	-	-

Results

Location	Tissue	µgC/cm²/h			$P_G:R_D$	$R_D:P_G$ %	EDW	gC/cm²/day
		P_G	R_D	P_N				P_N
Above canopy	Old lamina	0.3	0.2	0.1	1.5	66.7	614.5	-0.02
	New lamina	0.5	14.0	-13.5	0.04	2800	1227.4	-3.33
	Stipe	-	-	-	-	-	623.5	-
Below canopy	Old lamina	0.2	2.9	-2.7	0.07	1450	252.7	-0.68
	New lamina	0.3	4.9	-4.6	0.06	1633	425.5	-1.15
	Stipe	-	-	-	-	-	494.6	-

	µgC/mg dry wt/h	
Location	Holdfast	Stipe
Above canopy	0.004	0.015
Below canopy	0.002	0.002

Conclusions

All tissues were below the light compensation point; in particular, the new lamina tissue both above and below the canopy. The light irradiance above the canopy (0.87 cal/cm²/h) was of the same order as the light compensation point measured in the laboratory i.e. 0.5 cal/cm²/h (see Chapter 6). It cannot strictly be compared with the light compensation point measured for photosynthesis of tissues in the laboratory where only short term experiments are considered. The value of light compensation point found in the laboratory might be expected to be lower than that found in nature, where respiration is considered over 24 hours instead of the short term consideration in the laboratory. The irradiance recorded underwater in this experiment can be compared with the values found by Kain (1966) near Port Erin, Isle of Man. Thus the value of 0.87 cal/cm²/h is equivalent to 242 µg cal/cm²sec and Kain (loc cit) reported similar values at 4-5m BMLWS in February during a reasonable winter.

This experiment suggests that the growing algal tissue, i.e. the new lamina which was shown in Chapter 3 to have average dry weight of from 5 - 7g in February - probably cannot rely on photosynthesis as a source of carbon for growth during these early months.

Experiment L. March

Location Durness, Sutherland (58°33'N;04°45'W) Date 29.3.71 Daylength 11.7h

Conditions overcast; sea calm with surface ripples from strong wind

Temperature 6.1m - 7.5°C.

Details A one depth experiment at 6.1m with old lamina (OL), new lamina (NL), stipe (S), and holdfast tissue incubated above and below the forest canopy. No platforms were used and the jars were placed on a sand patch for open treatments and put on bedrock below the canopy; it was found that considerable sand movement had occurred during the course of the experiment and, in some cases, a layer of sand was present on the upper glass surface of the incubation jars so results may be spuriously low.

Integrator data

Location	corrected movement (mm/h)	% surface	cal/cm^2/h
surface	2.78	-	3.48
Above canopy	1.66	60	2.08
Below canopy	0.55	20	0.69

Results

Location	Tissue	µgC/cm^2/h					EDW	gC/m^2/day
		P_G	R_D	P_N	$P_G:R_D$	$R_D:P_G$ %		P_N
Above canopy	OL	2.7	4.1	-1.4	0.7	52	280.5	-0.66
	NL	8.8	4.2	4.6	2.1	47.7	290.8	0.05
	S	1.3	1.6	-0.3	0.8	123	401.7	-0.23
Below canopy	OL	1.7	6.4	-4.7	0.3	376	439.3	-1.33
	NL	4.5	6.7	-2.2	0.67	149	456.9	-1.07
	S	0.8	2.2	-1.4	0.36	275	550.3	-0.43

Location	µgC/mg dry wt/h Holdfast	Stipe
Above canopy	0.03	0.11
Below canopy	0.02	0.07

Conclusions

The experiment shows that of the growing tissue at this time only the new lamina above the canopy is showing positive net growth in terms of carbon uptake. The irradiance level above the canopy is about twice the February value and it is likely that the new lamina of canopy plants can photosynthesise all the carbon required for its growth. Stipe tissue both above and below the canopy is below compensation and it is possible that this tissue and the new lamina of plants growing under the canopy relies on translocates for growth.

Another example of the ability of the new lamina to photosynthesise adequately in March - at least in unshaded conditions - is seen from the extrapolated data for the new lamina from experiment B (page 89). These gave figures of :

4.6m	P_N = 1.2 gC/m^2 lamina/day	10.1m P_N = 0.49 gC/m^2 lamina/day
	P_G = 14.2 μgC/cm^2 lamina/h	P_G = 7.9 μgC/cm^2 lamina/h
	$P_G:R_D$ = 5.1	$P_G:R_D$ = 3.1

Experiment M April.

Location Fife Ness (56°17'N;02°36'W) Date 24.4.70 Daylength 14.11h

Conditions Bright; calm sea Temperature 3.1m - 6°

Details A one depth experiment with lamina, stipe, and holdfast tissue incubated with 10μci sodium bicarbonate-C14 above and below the canopy.

Integrator data

Location	corrected movement	% surface	cal/cm^2/h
surface	6.4	-	7.9
above canopy	2.03	32	2.54
below canopy	0.46	7	0.57

Results

Location	Tissue	μgC/cm^2/h			$P_G:R_D$	$R_D:P_G$ %	EDW	gC/m^2/day
		P_G	R_D	P_N				P_N
Above canopy	OL	13.5	2.4	11.1	5.6	17.8	208.4	1.33
	NL	10.1	1.8	8.3	5.6	17.8	189.7	0.99
	S	2.1	3.2	-1.1	0.7	152	803.0	-0.47
below canopy	OL	7.2	2.9	4.3	2.5	40.3	288.3	0.32
	NL	2.3	2.3	0	0	100	188.8	-0.23
	S	1.5	1.6	-0.1	0.94	107	341.1	-0.17

Holdfast tissue μgC/mg dry wt/h.

Location	Holdfast	Stipe
Above canopy	0.17	0.13
below canopy	0.03	0.17

Conclusions

By April it is apparent that the new lamina in shallow waters is capable of providing all its carbon requirements through its own photosynthetic capacity. The stipe tissue does not have this capacity either above or below the canopy. The situation of the jar on the below canopy platform may be rather unnatural since it was positioned on the bedrock whereas most plants below the canopy develop a new lamina some 8-15cm above this level where the light may be slightly better; this point is further discussed in the discussion in this chapter (see also Figure 2:1).

Experiment N MAY

Location Fife Ness Date 26.5.70 Daylength 16.21h.

Conditions Bright; calm sea Temperature 4.6m - 8.8°

Details A one depth experiment at 4.6m with lamina, stipe, and holdfast tissue above and below the forest canopy.

Integrator data

Location	corrected movement	% surface	cal/cm^2/h
surface	9.4	-	11.7
Above canopy	4.98	53	6.2
Below canopy	0.36	0.04	0.5

Results

Location	Tissue	µgC/cm^2/h					EDW	gC/m^2/day
		P_G	R_D	P_N	$P_G:R_D$	$R_D:P_G$ %		P_N
Above canopy	OL	14.6	3.6	11.0	4.1	24.7	234.1	1.50
	NL	17.3	4.0	13.3	4.3	23.1	262.0	1.84
	S	4.7	2.6	2.1	1.8	55.3	550.0	0.15
Below canopy	OL	5.9	4.2	1.7	1.4	71.2	272.5	-0.11
	NL	4.9	4.0	0.9	1.2	81.6	257.6	-0.16
	S	2.1	2.0	0.1	1.1	95.2	419.6	-0.13

Holdfast Tissue

Location	µgC/mg dry wt/h Holdfast	Stipe
Above canopy	0.08	0.30
Below canopy	0.01	0.19

Conclusions

The results from this experiment indicate that by May all tissues including the stipe tissue may produce enough carbon for growth by their own photosynthetic activity. Lamina and stipe tissue below the canopy are all below the compensation point for photosynthesis.

Experiment O JUNE

Location Durness Date 22.6.71 Daylength 17.49h

Conditions Bright; calm sea Temperature 5.5m - 10.2°

Details A one depth experiment near Loch Sian (Grid Ref: NC 449628) in Loch Eribol. There was no old laminae left at this time so new lamina (NL) stipe (S), and holdfast tissues were used. Sections were incubated in C14 labelled seawater as before.

Integrator data

Location	corrected movement	cal/cm^2/h
*surface		19.5
Above canopy	3.1	3.9
Below canopy	-	-

* Integrator did not work: irradiance from I.S.C.O. Spectoradiometer reading at that time.

Results

Location	Tissue	µgC/cm^2/h			$P_G:R_N$	$R_D:P_G$ %	EDW	gC/m^2/day
		P_G	R_D	P_N				P_N
Above canopy	NL	7.3±2.1	2.9±0.55	4.4	2.5	39.7	187.2	0.62±0.24
	S	1.5	2.1	-0.6	0.7	140	353.6	-0.24
Below canopy	NL	1.0±0.3	2.3±0.1	-1.3	0.5	230	151.3	-0.37
	S	0.1	2.7	-2.6	0.04	2700	687.8	-0.63

µgC/mg dry wt/h.

Location	Holdfast	Stipe
Above canopy	0.03	0.12
Below canopy	0.01	0.03

Conclusions

In June the lamina tissue shows positive net carbon uptake above the canopy layer but in the case of stipe tissue it seems that photosynthesis cannot support growth. The great shading effect of the canopy can again be seen for the results of the below canopy jars.

Experiment P JULY

Location Fife Ness Date 8.7.71 Daylength 16.88h

Conditions Good; calm sea Temperature 3.1m - 12°
9.1m - 11.5°

Details A two depth experiment at 3.1m and 9.1m using lamina discs only to investigate the photosynthetic capacity at this time of year and to simultaneously measure photosynthesis with the Cl^4 technique and the Winkler oxygen method. This was done to check whether the Cl^4 technique measures gross or net photosynthesis (see introduction to this chapter). Thus 5 jars were set up at 3.1m; 2 lamina discs were put into 2 darkened jars, 2 more were incubated in the light, and the remaining jar was used as a dark control with no tissue in.
Two light and two dark jars were set up at 9.1m.

Integrator Data (no surface data)

Depth (m)	corrected movement (mm/h)	$cal/cm^2/h$.
3.1	4.20	5.2
9.1	0.53	0.7

Winkler analysis

The dark control jar gave the initial oxygen content of the seawater and at the end of the experiment a water sample was taken from each jar by carefully sealing a McArtney bottle underwater in each jar so no air bubbles were left in the bottles.

Reagents

I. $MnCl_2 . 4H_2O$ Manganous chloride. 40g in 100ml H_2O

II. KI + KOH - 'alkaline iodide'. 60g of KI and 30g of KOH in 100 ml H_2O.

III. 50% H_2SO_4

IV. $Na_2S_2O_4$ - 0.04N sodium thiosulphate solution.

V. Soluble starch.

Procedures

Two small holes were made in the screw tops of 28ml bottles so that reagents could be injected into one hole with a relief syringe in the other hole

taking out the displaced medium. It was shown from dye studies (Drew, pers. comm.) that no chemicals went into the relief syringe.

The $MnCl_2$ (1.0ml) and alkaline iodide (1.0ml) were added first when a precipitate was given. This was shaken hard and 1.0ml of H_2SO_4 was added to liberate the iodine from the manganese complex. The contents were transferred to 50ml conical flasks and sodium thiosulphate was added slowly until the precipitate went a little clearer. Starch indicator was added and more thiosulphate was added dropwise until the blue colour disappeared. The volume of thiosulphate added was noted.

Calculation

The volume of $MnCl_2$, alkaline iodide and H_2SO_4 added, (3ml) was subtracted from the volume of the 28ml screw top bottles.

$$1ml\ 0.04N\ Na_2S_2O_3 = 350\ \mu l\ O_2$$

Thus the oxygen content was worked out from the volume of thiosulphate added. The initial oxygen content was estimated from the dark control with no tissue.

Results

Depth	Treatment	$\mu lO_2/h$	$\mu lO_2/mg$ dry wt/h	$\mu lO_2/cm^2/h$	*$\mu gC/cm^2/h$
3.1m	Light	155	0.4	4.7	2.31
	Dark	-86	0.4	2.7	1.33
9.1m	Light	167	0.95	5.0	2.48

*Conversion from $\mu l\ O_2$ to μ gC using the factor obtained in the following way:

$$\text{Photosynthetic quotient} = \frac{O_2 \text{ evolved}}{CO_2 \text{ absorbed}} = 1.08$$ (assuming mannitol is primary photosynthate)

$$x\ \mu lCO_2 \text{ absorbed} = \frac{y\ \mu lO_2}{1.08}$$

$$1\ \mu lCO_2 \text{ contains } \frac{44}{22.4}\ \mu g\ CO_2 \text{ at NTP}$$

$$\therefore\ x\ \mu lCO_2 \text{ contains } \frac{44.y}{22.4 \times 1.08}\ \mu g\ CO_2$$

$$\therefore\ \mu gC \text{ in } x\ \mu lCO_2 = \frac{12.44.y}{44 \times 22.4 \times 1.08}$$

$$= \underline{0.496xy\ \mu gC}.$$

The values obtained in the light bottles represent net photosynthesis and gross is obtained by adding on the dark control values.

Winkler net and gross synthesis.

Depth (m)	$\mu gC/cm^2/h$ P_N	P_G
* 3.1	2.31	3.64 ± 1.07
9.1	2.48	3.81 ± 0.298

* The low value obtained at 3.1m was probably due to loss of some of the water sample on analysis.

Cl4 Data

Depth	$\mu gC/cm^2/h$ P_G	R_D	P_N	$P_G:R_D$	$R_D:P_G$ %	EDW	$gC/m^2/day$ P_N
3.1	4.0 ± 3.4	4.9 ± 1.3	0.9	0.8	123	341.2	-0.88 ± 0.49
9.1	3.2	2.7 ± 0.4	0.5	1.2	84	186.2	-0.12

Conclusions

Firstly the close correlation between the value for gross photosynthesis obtained from the Winkler analysis and that obtained from the Cl4 analysis at 9.1m would seem to justify the assertion in the introduction of this chapter that Cl4 uptake measures gross photosynthesis. Sěstâk et al. (1971) point out the importance of checking the Cl4 with an independent method.

Secondly, from the Cl4 data it is apparent that photosynthetic activity is falling off in July at Fife Ness and the lamina tissue is below compensation point at both depths. Experiments C,D,E describedin section 1 of this chapter found that net photosynthesis of the lamina was also low during July in Cornwall. (N.B. See Addendum, page 206 ; a further point is that, if the respiration correction used for the Cl4 data is spuriously high, then the values of net photosynthesis found by the Cl4 technique will be more comparable to those found by the Winkler method).

DISCUSSION

The photosynthetic capacity of the kelps estimated in these experiments can be compared with the data for other terrestrial and sublittoral species. Thus in Table 5:3 the net photosynthesis of various species under natural conditions is tabulated.

TABLE 5:3

Net Photosynthesis (natural conditions)

Species	Light $cal/cm^2/h$	Temperature °C	P_N ($\mu gC/cm^2/h$)	Author
Pyrus malus	natural	NS	54.6	Spector, 1956
Vicia faba	"	"	46.4	"
Pinus taeda	"	"	39.0	"
Solanum tuberosum	25.2	"	1.4	Chapman & Loomis, in Thomas, 1955.
Macrocystis pyrifera	natural	15	39.6	Clendenning & Sargent, 1957
Saccorhiza polyschides	5.1	13.1	15.8	Exp. H, this chapter
Laminaria hyperborea	6.2	8.8	13.3	Exp.N, this chapter
Laminaria saccharina	natural	9.6	13.8	Exp.G, this chapter

It can be seen that the photosynthesis of sublittoral macrophytes compares favourably with other species. This is probably due to the high photosynthetic efficiencies and the high light trapping ability of the kelps. The high photosynthesis of Macrocystis pyrifera and the high photosynthesis to respiration ratios (from 20 to 40, Clendenning & Sargent, 1957) indicated a high productive capacity in this species (McFarland & Prescott, 1959).

Figure 5:4 shows a compound graph of all the old and new lamina gross photosynthesis values of L. hyperborea and the lamina values of Saccorhiza polyschides at the various light orradiances recorded. This graph does not take into account any seasonal variation in the data but the dates of some of the values are put in the graph.

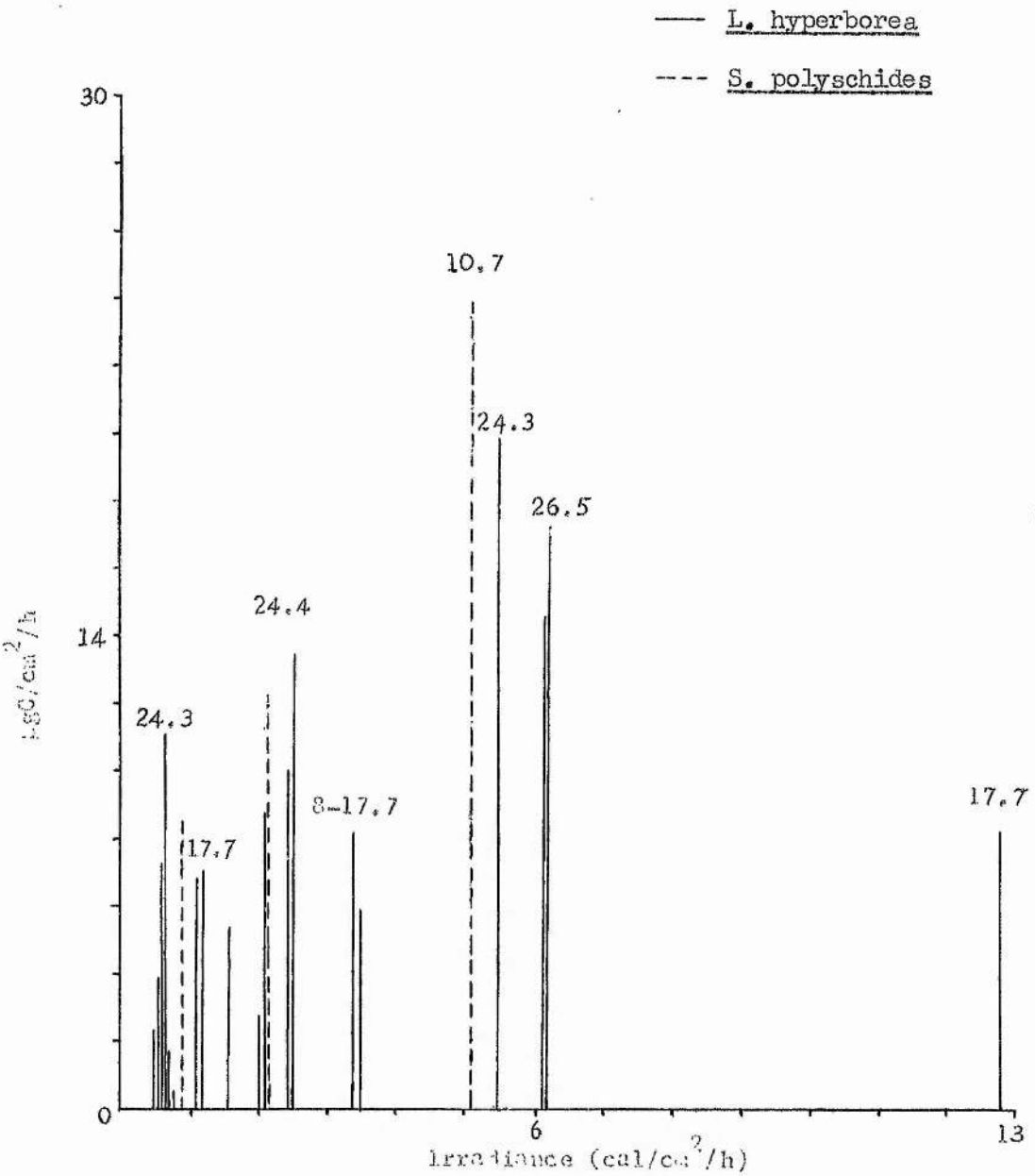


Figure 5:4 Gross photosynthesis of L. hyperborea and S. polyschides at various light intensities.

The 'euryphotic' nature of Laminaria ochroleuca has been commented upon by Pérès (1967) and Fredj & Giermann (1968) and L. hyperborea appears to show a similar ability to photosynthesise well over a wide range of light intensities. This is in accord with the wide depth range of this species (Kain, 1967). The cluster of values at the low light values (0.5-4.0 cal/cm^2/h) are from experiments in February, March and April but there are some values for July. There is some indication that these values are lower than expected. A decline in photosynthesis has been suggested by Black & Dewar (1949) to explain the slowing up in the growth rate during the summer months. This point is taken up again when seasonal aspects of photosynthesis are discussed.

The high values for lamina photosynthesis of L. hyperborea at 5-6 cal/cm^2/h are correlated with season. These results are for March and May and indicate this is the optimum time for photosynthesis. The reduced value of photosynthesis at 12.8 cal/cm^2/h may be correlated with either season (July data) or possibly with a light inhibition. There was no indication of a light inhibition of photosynthesis in the shallow incubations (section 1) but it is possible that these incubations were below a photo-inhibitory zone. Black (1950b) attempted to correlate the changes in chemical content, in particular mannitol, with photosynthesis at various depths and concluded that the maximum photosynthesis of L. hyperborea occurred at 6-10m and not at the surface. Whittick (1969) found that the biomass of upper age class plants of L. hyperborea increases from low water mark to 6m but correlated the reduced lamina biomass in the first few metres with physical damage from wave action. There is some indication from the extracted dry weight data in Experiments C,D (pages 91, 93) that the unit weight of lamina increases to a maximum at 10.7m which confirms the view that maximum biomass may not be just below the surface. Drew (1969b) found a photo-inhibition of photosynthesis in Peyssonelia in the high irradiances of surface Mediterranean waters. More precise measurements of photosynthesis of L. hyperborea over the depth range are required to investigate this aspect.

The photosynthetic capacity of the holdfasts could not be satisfactorily expressed on an area basis to obtain net production data but on a weight basis the fixation appeared to be low compared with that of stipe tissue. Johnston (pers. comm.) has found that chloroplasts in the haptera are poorly developed and the data presented here would suggest a low photosynthetic capacity in these tissues.

Figure 5:2 showed that photosynthesis, although reduced with depth, did not decrease to the same extent as the logarithmic reduction in light irradiance over the same depth range. It was also found that the efficiency of utilization of available light increased with depth both for L. hyperborea and S. polyschides. John (1971) found that the net efficiency of energy fixation in L. ochroleuca and S. polyschides increased with depth to a peak value of 2% at 8.4m, and decreased below this depth. The high photosynthetic efficiencies reported here may be due to the strong absorption maximum of fucoxanthin for the predominantly green light underwater (Haxo & Blinks, 1950) and the ability of this accessory pigment to transfer light energy to chlorophyll (Wassink & Kersten, 1946; Tanada, 1951, Haxo, 1960). The high light trapping ability of Laminaria may also be a result of a relative lack of opaque cell walls and intercellular air spaces, which reflect and absorb much light, compared with terrestrial plants (Strain, 1950).

It was noted in chapter 3 that, in some cases, the performance of individual L. hyperborea plants is not significantly reduced over the depth range from 3.1 to 9.1m. Kain (1971) found that the growth rate of individual plants was similar over a wide range of depth from 4 to 22m below mid-tide. The fact that reasonable growth is maintained by plants over such a wide depth range suggests that the species is well adapted to survive varying light regimes.

Drew & Larkum (1967) and Drew (1969) have reported large increases in photosynthetic efficiencies of several sublittoral marine macrophytes with increasing depth. The increased efficiencies could not be ascribed to changes

in thallus weight per unit area or to changes in chlorophyll concentration. Adaptations, if they existed, were rapid since transplants showed no difference in photosynthesis rates to control during 4 hour experiments.

Björkman & Holmgren (1963) found that plants from shaded habitats had higher photosynthetic capecity and utilization of light under low light conditions than plants from exposed habitats. The increased efficiencies found were attributed to properties of the plants which increase light absorption. It was suggested that changes in leaf anatomy may be of importance but the slightly higher amounts of chlorophyll per leaf area probably did not have much effect on light absorption. The possible basis for the increased efficiencies found in L. hyperborea is discussed in chapters 6, 7, and 8 where factors such as lamina thickness, pigment concentrations, and respiration are assessed as adaptive mechanisms. It should also be noted that S. polyschides shows a change in lamina morphology with depths and Norton & Burrows (1969a) have correlated the presence of a thin lamina with the lack of wave action. The increased lamina area at depth may, however, also give rise to an increased absorption of light or, perhaps, more important, the thinner lamina at depth may result in a reduced 'respiratory load' on the photosynthetic layer.

In several experiments it was noted that the forest canopy exerts an intense shading effect on the photosynthesis of tissues placed below it. The light levels under the canopy varied from 0.04 to 20% of the surface value and from 7 to 33% of the values above the canopy. Variations are undoubtedly due to differences in the density of the forest. Kitching (1941) found that the forest canopy can cut off up to 99% of the ambient light. The results from the C^{14} experiments may be low as the incubation platforms were placed on the bedrock with the upper surface of the jars some 18cm above the bedrock and the lamina discs only about 10cm above the bedrock. As Figure 2.1 indicates, the position of incubation is often below the level of the developing lamina and stipe which may be some 50cm above the bedrock. It is possible that the frequent movements of the laminae

in the canopy give rise to 'sunflecks' which could allow some photosynthesis over intermittent periods. Evans (1966) found that sunflecks of 33% of full light covered about 2% of the floor of a terrestrial forest when the sun was at its zenith, and lasted in one spot for a total of about 7 minutes. It is possible the same situation exists in the Laminaria forest. McFarland & Prescott (1959) have found the shading capacity of the Macrocystis forest to be of the same magnitude as a tropical rain forest.

The large shading effect of tne canopy on the photosynthesis of S. polyschides seen in experiment I provides support for the suggestion of Norton & Burrows (1969a) that the L. hyperborea canopy may be an important factor inhibiting the development of S. polyschides and limiting its upward extension.

The experiments with L. saccharina suggested a high photosynthetic capacity. That occassionally a negative value of net photosynthesis is given in June was also found by Tikhovskaya (1940), but positive values were obtained below 10m on one occasion.

The result of experiment J suggested that the epibiota had little effect on photosynthesis in L. hyperborea. Clendenning & Sargent (1958) found that Membranipora has little effect 'per se' on the photosynthetic capacity of Macrocystis but found that the compensation point was raised in the encrusted kelp complex. It is likely that the epibiota may have a significant effect at or near compensation points either at depth or under the canopy.

Figure 5:5 compares the photosynthesis rates in June and July of the 3 sublittoral species. It is suggested that some correlation exists between longevity and metabolic status. It does seem that a reduction in photosynthetic capacity occurs from the annual, fast growing S. polyschides to L. saccharina with a life span of 3 years (Parke, 1948), and finally to smaller rates found in L. hyperborea, which can live up to 13 years. The increased metabolic rate in the less long-lived competitors of L. hyperborea would allow rapid development where gaps occurred in the forest. John (1971) has shown that S. polyschides has the highest net productivity yet found in sublittoral marine macrophytes.

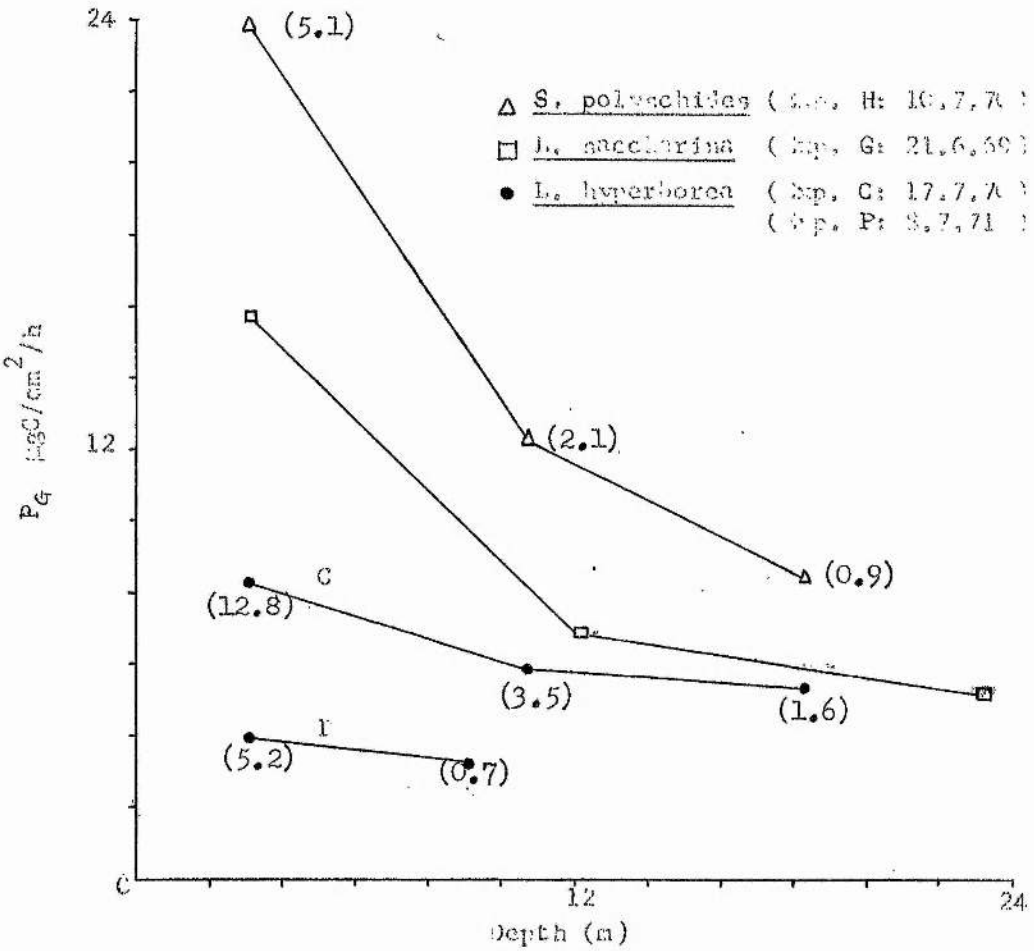


Figure 5:5 Comparison of rates of photosynthesis at various depths of the main members of the kelp forest ecosystem

The irradiance in cal/cm^2/h found at each depth is shown in brackets.

The seasonal measurements of photosynthesis can be used to analyse the energy flow in the ecosystem. Some assessment can be made of the important environmental factors controlling seasonal growth and the productivity data from the Cl4 data can be compared with that from the biomass data. In Figure 5:6 below the various values from the experiments described for gross photosynthesis and respiration of the new lamina above the canopy, are presented as histograms with the corresponding values of the $P_G:R_D$ ratio, the sea temperatures and light data for the months of the year. It must be emphasised that the respiration rates (carbon losses) are largely a reflection of the biomass of tissue used since the rate is found for the total dry weight present before correcting for area (see Introduction in this Chapter). Thus the value in February is high because a large amount of tissue

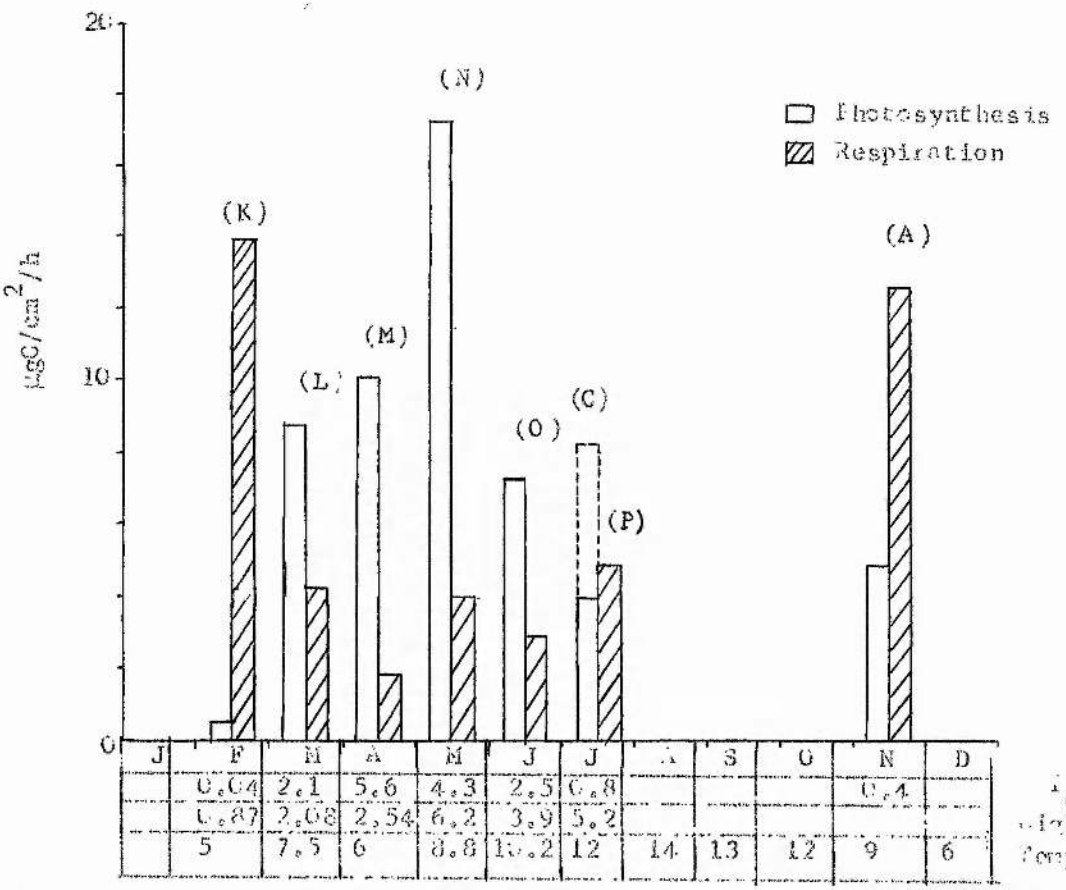


Figure 5:6 Photosynthesis (P_G) and respiration (R_D) of lamina tissue of L hyperborea above the canopy (3.1m) during the season.

was present. In this case the disc was cut out of the small new lamina available then and may have included parts of the thick meristematic region.

It can be seen from this figure that photosynthesis is negligible in February with a low ratio of photosynthesis to respiration and this indicates that the average of 5g dry weight of new lamina produced by February (for canopy plants only) must have come from a source of carbon other than that produced from photosynthesis. It will be shown in chapter 9 that translocation of reserves from the old lamina assists this early growth in the 'lag' phase.

In March it is evident that light has improved sufficiently to allow positive net photosynthesis. The low temperature of the sea at this time keeps the respiratory loss low. As the light improves photosynthesis increases rapidly and a peak of photosynthesis is found in May when the light was at a maximum and there was still only a small respiration loss as lamina biomass was low. The dynamics of this rapid lamina growth phase resembles the pattern of high photosynthesis at low temperatures in marine macrophytes formulated in the Harder:Kniep theory (see Gessner, 1955) which explained the seemingly paradoxical predominance of large algae in colder, turbid waters on the basis of the ability of these species to photosynthesise well at low temperatures and light intensities. Lampe (1935) qualified the theory and held that the ability of a species to adapt to changes in temperature whilst still maintaining a high photosynthetic capacity was important. Tikhovskaya (1940) noted the low temperature and light optima for net photosynthesis in L. saccharina and found a maximum photosynthesis to respiration ratio in February to May. The ability of Macrocystis to adapt to progressively lower temperatures without loss of photosynthetic capacity was commented on by Clendenning & Sargent (1958). Photosynthesis was found to decline in June even though light irradiance underwater was still at a high level. Thus, in July negative values for net photosynthesis were given and this corresponds to the slow growth phase. No further experiments

were carried out between July and November but the value of gross photosynthesis in November exceeds that found in July so it is possible that a second burst in photosynthesis occurs in the autumn akin to the second phytoplankton bloom. It must be emphasised that there are geographical variations in these rates and the value for gross photosynthesis for experiment C in Cornwall is super-imposed with the value for Fife Ness in July (experiment P).

Tikhovskaya (1940) found for L.saccharina that the reduced P : R ratio in the summer was the result of reduced photosynthesis and increased respiration and noted that the temperature in the summer was at a maximum and nutrients were at the lowest value for the year. Tseng et al. (1957) showed that summer temperatures and nutrients were critical in attempts to transplant L japonica. Clendenning & Sargent (1958) correlated decline of photosynthesis of Macrocystis in the summer with high temperatures and Sundene (1962, 1964) also correlated the summer decline in the growth of L. digitata with increased temperatures.

Black (1949), Black & Dewar (1949), and Gessner (1955) have correlated the seasonal growth and summer decline in macrophytic algae with the nutrient status in the sea. It is well known that the abundance of phytoplankton is largely controlled by nutrient concentrations in the sea (Harvey, 1928). Marshall & Orr (1948) found that attached marine algae could take up added fertiliser in a sea loch to the detriment of the phyto-plankton. No nutrient addition experiments were carried out so that there is no evidence to correlate the drop in photosynthesis in the summer with nutrient depletion in coastal waters. The fact that nutrients fall rapidly from March to August (Harvey, loc cit) whilst there is a rapid rise in photosynthesis from March to May suggests that nutrients may be depleted by L. hyperborea as well as phytoplankton and summer thermoclines may 'lock up' nutrients in deeper water.

On the other hand, Bidwell (1958b) found that varying levels of nutrients had little effect on the photosynthesis of Fucus vesiculosis.

Lüning (1970a) suggested that nutrients have little effect on the delayed reduction in growth rate of the new lamina of L. hyperborea at 6m depth compared to the 2m samples since strong tidal currents would make it unlikely that the nutrient status at 6m was much different than that at 2m.

No explanation can be put forward here as to the cause for the reduced photosynthesis rate in the summer but the overall slowing of biomass increment from June to October must be the result of an increased respiratory 'load' on the photosynthetic tissues. It is unlikely that increased temperatures result in a greatly increased respiration rate since data in Chapter 7 (see Figure 7:1), suggests that temperatures above 10°C cause a decrease in the rate of respiration which may be the result of direct damage to enzyme systems. It is thought that the increased respiratory rate up to 10°C and the accumulated lamina biomass reach a limit for the photosynthetic mersitoderm to support in the summer and consequently growth slows. That photosynthesis is not always reduced in the summer is shown in Figure for the Cornwall data (experiment C) and Black (1950a,b) suggests that the continued but 'restricted' growth of L. hyperborea from July to December leads to an accumulation of laminarin presumably through photosynthesis), whilst species such as L. digitata and L. saccharina which still continue to grow during the summer do not accumulate laminarin. Thus photosynthesis is not totally curtailed in the summer.

A comparison can be made between the rates of net primary productivity from the C14 method and the net assimilation rates obtained from biomass increment studies described in Chapter 3.

The data for NAR and net photosynthesis (gC/m^2 lamina/day) for each month are set out in Table 5:4 .

Table 5:4 NAR v Cl4

(gC/m^2 lamina/day)

Month	January	February	March	April	May	June	July	August	Sept.
NAR	7.63	2.15	1.04	0.61	1.23	0.32	0.08	0.07	0.06
Cl4	0	-3.33 (K)	0.05 (L) 1.20 i (B)	0.99 (M)	1.84 (N)	0.62 (O)	-0.88 (P) 0.70 ii (C,D)	-	-

The Cl4 data is from the following localities:-

Experiments K, M, N, P - Fife Ness

Experiments L, O - Durness

i ( ) - Experiment B - extrapolated new lamina data for 4.6m - Arisaig.

ii( ) - Experiments C, D - mean of new lamina P_N from 3.1m - Mousehole.

The data for January and February are misleading in the sense that they do not represent photosynthetic production but possibly represent the increment from translocation. They have been obtained by dividing a small biomass increment by an even smaller value of LAI so that the values are large.

When the data for the growing months of March to July are compared it can be seen there is quite good agreement between the two methods of measuring primary productivity. It must be held in mind that the Cl4 experiments were often carried out in different environments from that at Arisaig where the biomass data was collected. Thus, for example, a negative value for net photosynthesis is given by the Cl4 estimate for July at Fife Ness, whereas the Cornwall data (Mousehole) showed a positive value. These variations are probably due to geographical and climatic differences. The extrapolated value for Arisaig in March from experiment B shows good agreement with the NAR for that month. Other Cl4 experiments show higher values for net production than the corresponding NAR.

This is probably due to the fact that the C14 experiments were carried out on reasonably clear days and represent production extrapolated from short term experiments over the middle part of the day when, with the sun at its zenith, maximum penetration of light into the sea occurs. The NAR from biomass data, however, is an average over a month. The two values are very comparable indicating that these two independent methods can be used with some confidence to measure productivity.

CHAPTER 6

Photosynthesis measurements at various light intensities

The experiments described in this chapter were carried out in order to obtain data on the light compensation and saturation points for the photosynthesis of new and old lamina tissue of L. hyperborea. This data could then be used in in conjunction with the C14 experiments described in Chapter 5 to assess the performance of the alga at various depths and during the growing season.

Experiment 6:1 Photosynthesis:light intensity curve for the old lamina of a shallow plant.

Details The experiment was carried out on 8.12.70. 7.5cm^2 discs of old lamina tissue from a plant collected from 3.1m at Fife Ness were incubated in 28ml screw capped bottles with 1μCi of sodium bicarbonate-C14. Two bottles were placed in each incubation chamber. The following light regimes were used:- Full light (4.98 cal/cm^2/h), 33% of full light (1.66 cal/cm^2/h), 10% of full light (0.498 cal/cm^2/h), and 1% of full light (0.05 cal/cm^2/h). Samples were incubated for 2 hours 43 minutes at 10°C, killed in 80% ethanol and counted. The results are set out in Table 6:1.

Table 6:1

Photosynthesis of the old lamina at various light intensities

Percentage full light	Irradiance cal/cm^2/h.	P_G	μgC/cm^2/h R_D (i)	R_D (ii)	P_N (i)	P_N (ii)	mg. Extracted dry wt.
(100%)	4.98	6.4±0.35	2.7	3.6	3.7	2.8	52.95
(33%)	1.66	6.3±1.1	2.5	3.3	3.8	3.0	49.8
(10%)	0.498	2.6±0.1	2.5	3.4	0.1	-0.8	41.4
(1%)	0.05	0.6±0.3	2.1	2.8	-1.5	-2.2	48.3

where P_G = gross photosynthesis
P_N = net photosynthesis
R_D = dark respiration
(i) 5°C : respiration rate (as carbon loss) = 0.38 μgC/mg dry wt/h
(ii) 10°C : respiration rate = 0.51 μgC/mg dry wt/h

(The respiration rates were obtained by inter-polation of the respiration/temperature graph in Chapter 7). See Addendum, page 206.

Calculation of light compensation and saturation points

The value of gross photosynthesis at each light irradiance was plotted against irradiance in Figure 6:1. The compensation irradiance is defined as the irradiance where no net photosynthesis occurs. At this point the gross photosynthesis is balanced by respiration so the intercept of a line drawn to the irradiance axis from a point on the curve equivalent to the respiratory rate gives the compensation point. It is assumed that at low light intensities the rate of photosynthesis is not affected much by temperature and Warburg (1919) found that the Q_{10} of chemical reactions at low light intensities was 1, indicating that the control of rates was entirely photochemical. Thus the compensation point for lamina tissue at 5°C has been interpolated from this curve which was determined at 10°C.

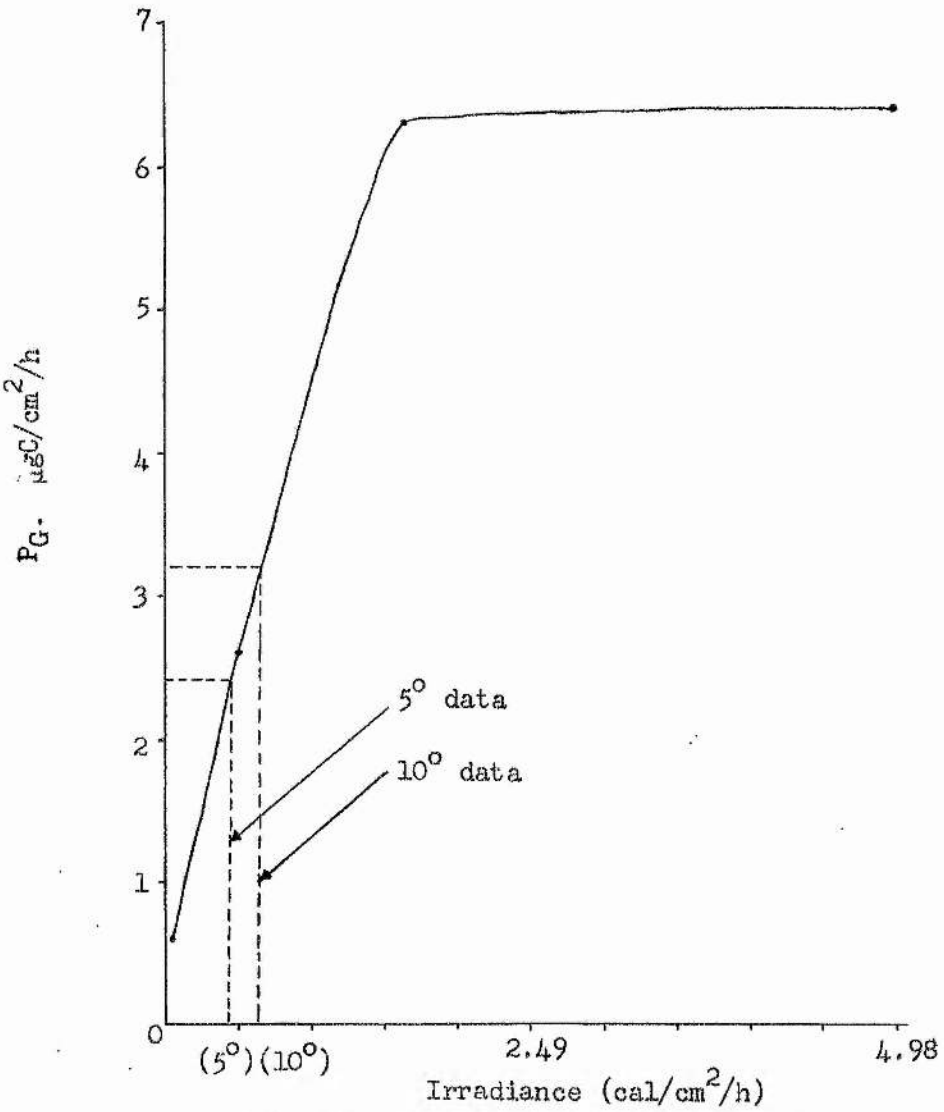


Figure 6:1 Photosynthesis:light intensity curve for the old lamina

(i) Compensation point at 5°C

The mean value of respiration rate at 5°C was 2.4±0.46 μgC/cm^2/h and the intercept of this point on the graph with the light intensity axis gives a value of 0.45 cal/cm^2/h (125 μg cal/cm^2/s).

(ii) Compensation point at 10°C.

The mean value of respiration at 10°C was 3.2±0.6 μgC/cm^2/h and the intercept on the light intensity axis gave a value of 0.65 cal/cm^2/h. (180 μg cal/cm^2/s).

(iii) Saturation light irradiance

The lamina tissue appeared to be saturated at 33% of full light intensity so the saturation irradiance was calculated as 1.66 cal/cm^2/h (461 μg cal/cm^2/s).

Photosynthetic efficiencies

The efficiency of utilisation of the light at each irradiance is shown in Table 6:2.

Table 6:2

Photosynthetic efficiencies of old lamina

Irradiance (cal/cm^2/h	P_G (μgC/cm^2/h	P_G (cal/cm^2/h	Photosynthetic efficiency (PE)	
			total light	50%
4.98	6.4	0.064	1.29	2.58
2.49	6.37	0.064	2.57	5.14
1.66	6.3	0.063	3.8	7.6
0.498	2.6	0.026	5.22	10.44
0.249	1.45	0.015	6	12

Using calorific content of carbon as 0.01 cal/μg carbon (Table 3:1)

Experiment 6 ii Photosynthesis:light intensity curve for the new lamina tissue

Details The experiment was carried out on 13.5.71. A similar experiment to 6i was carried out with the new lamina tissue from a plant collected from 4.5m at Fife Ness to estimate compensation and saturation irradiances. The temperature was 8.5°C. The results are set out in Table 6:3 and in Figure 6:2.

Table 6:3

Light intensity cal/cm^2/h	P_G	R_D (5°C) µgc/cm^2/h	P_N	Extracted dry weight (mg)
4.98	0.4	2.4	-2.0	62.9
1.66	0.6	2.4	-1.8	68.1
0.498	2.4	2.4	0	52.5
0.05	-	2.4	-	50.1

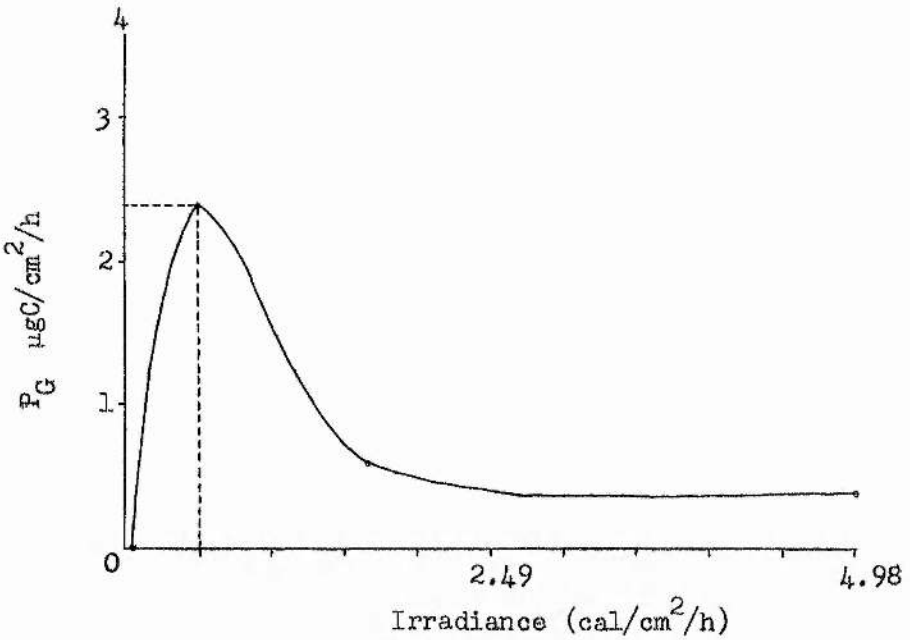


Figure 6:2 Photosynthesis:light intensity curve for the new lamina

(i) Compensation point at 5°C

From the PI curve the intercept of the respiration rate (2.4 µgC/cm^2/h on the light irradiance axis gives a value of 0.498 cal/cm^2/h (138 µg cal/cm^2/s). This value is very similar to that found for the old lamina.

(ii) Saturation point of the new lamina

The rate of photosynthesis in the new lamina appears to fall off with increasing irradiance and the saturation point coincides with the compensation point at 0.498 cal/cm^2/h. (138 µg cal/cm^2/s).

Photosynthetic efficiencies

The efficiency of utilisation of each irradiance is given in Table 6:4.

Table 6:4

Photosynthetic efficiencies of new lamina

Irradiance (cal/cm^2/h)	P_G (µg/cm^2/h	P_G (cal/cm^2/h)	PE total	PE 50%
4.98	0.4	0.004	0.08	0.16
1.66	0.6	0.006	0.36	0.72
0.498	2.4	0.024	4.82	9.64
0.249	1.7	0.017	6.83	13.65

Experiment 6 iii Photosynthesis:light intensity curve for a deep growing plant

Date 10.7.71

Details This experiment was carried out on 10.7.71 and was performed to see if the deep growing plants of L. hyperborea have increased efficiencies at low irradiances compared with the photosynthetic efficiency of shallow plants at the same irradiances and also to compare the compensation intensity with that for plants from shallow water.

The specimen for this experiment was obtained from 9.1m at Fife Ness and was kept in dim light in the aquarium until used. The experimental conditions are as for experiment 6i. The temperature was 13.5°C.

Results The results are shown in Table 6:5 and Figure 6:3.

Table 6:5

Photosynthesis of deep growing lamina at various light intensities

Irradiance (cal/cm^2/h)	P_G	R_D µgC/cm^2/h		P_N		Extracted dry wt.
		(i)	(ii)	(i)	(ii)	
4.98	6.3±1.3	1.85	2.5	4.5	3.8	36.9
1.66	3.4±0.4	1.95	2.7	1.5	0.7	38.8
0.498	2.5±0.2	1.6	2.2	0.9	0.3	32.1
0.05	-	1.65	2.2	-	-	32.4

(i) respiration rate at 5°C

(ii) respiration rate at 10°C

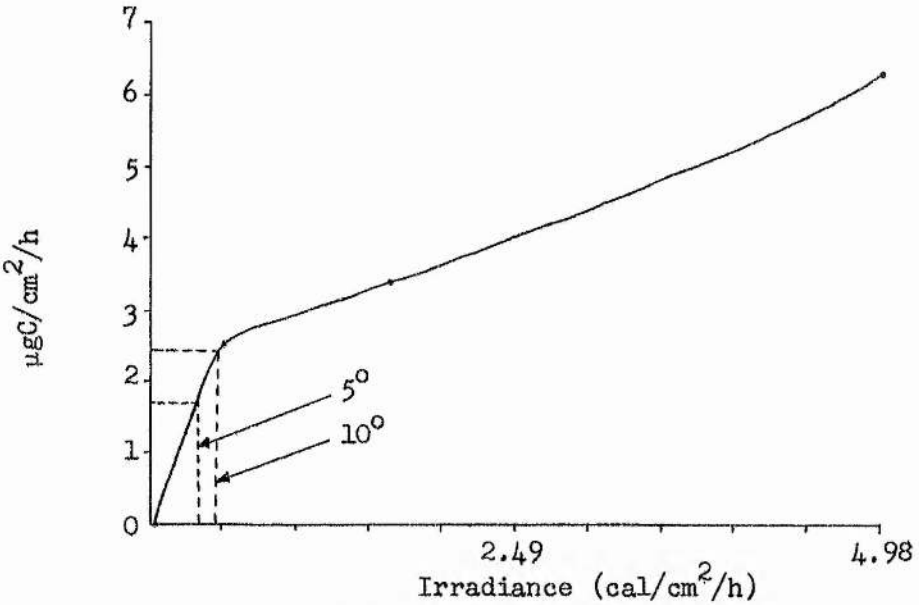


Figure 6:3 Photosynthesis:light intensity curve for deep plant

(i) Compensation point at 5°C

Using the mean value of respiration rate at 5°C of 1.76 μgC/cm^2/h the intercept of this point on the PI curve shown in Figure 6:3 gives a value for compensation irradiance of 7% of 4.98 cal/cm^2/h: this value - 0.35 cal/cm^2/h (97 μg cal/cm^2/s) - is somewhat below that found for the old and new lamina of shallow plants (0.45 and 0.498 cal/cm^2/h respectively).

(ii) Compensation point at 10°C

The mean value of respiration at 10°C was 2.39 μgC/cm^2/h and the intercept of this point on the irradiance axis gave a compensation point of 0.47 cal/cm^2/h (130 μg cal/cm^2/s); again this is somewhat below the value obtained for the compensation point at 10°C for the old lamina tissue of shallow plants (0.65 cal/cm^2/h).

(iii) Saturation point

The PI curve for deep plants does not seem to level off with increasing light intensity so no accurate value could be obtained; however, a shoulder is given at 0.498 cal/cm^2/h and the photosynthesis rates are quite low up to 4.98 cal/cm^2/h.

Photosynthetic efficiencies

The efficiency of utilization of each irradiance is given in Table 6:6

Table 6:6

Photosynthetic efficiencies of deep growing lamina

Irradiance (cal/cm^2/h)	P_G (μgC/cm^2/h)	P_G (cal/cm^2/h)	PE	
			total	50%
4.98	6.3	0.063	1.27	2.54
2.49	4.0	0.04	1.61	3.22
1.66	3.4	0.034	2.05	4.1
0.498	2.5	0.025	5.02	10.04
0.249	1.3	0.013	5.22	10.44

Discussion

The compensation irradiances found for lamina tissue of L. hyperborea of 0.35 to 0.498 cal/cm^2/h at 5°C, and 0.47 to 0.65 cal/cm^2/h at 10°C, are not especially low compared to other macrophytic algae. Kanwisher (1966) found the compensation point for Chondrus to be 25 foot-candles. The foot-candle is equivalent to about 0.004 cal/cm^2/h so this works out to 0.09 cal/cm^2/h. Bunt (1964) found the compensation point of ice micro-algae to be about 2.5 ft.-candle (0.01 cal/cm^2/h).

However, the saturation irradiances are low compared with the value of 5.3 cal/cm^2/hr. (1100 ft-c) for Macrocystis (Clendenning & Sargent, 1957).

Kain (1966) quotes some unpublished data suggesting the saturation level for photosynthesis for old lamina tissue was about 400 μg cal/cm^2/s. This agrees with the saturation level found here of 461 μg cal/cm^2/s and Kain (loc cit) also suggested the saturation level in the young lamina (i.e. the new lamina) was much lower than the value for the old lamina and the data quoted here confirms this, where a light saturation level of 0.498 cal/cm^2/h (138 μg cal/cm^2/s) was found for the new lamina.

On considering the efficiency of utilisation of the various irradiances several features emerge. It is apparent that the old lamina of shallow plants is more efficient at utilising all the light intensities compared with the deep plants. Thus it appears that the deep plants are not more efficient at lower light intensities compared with shallow plants as found by Björkman & Holmgren (1963) for low light and high light intensity adapted plants. The reduced compensation point given at depth is largely due to the reduced respiratory loss. This reduction in respiration at depth in turn, is largely a function of the smaller biomass of lamina since respiration is worked out on a weight basis before converting to an area basis. If the shoulder on the PI curve for deep plants at 0.498 cal/cm^2/h represents the saturation intensity then the lowered saturation level of deep plants compared with shallow plants is similar to the findings of Steeman-Nielsen et al. (1962), where low light grown Chlorella was

saturated at a lower light intensity than high light grown Chlorella.

On comparing the results for the new lamina and the old lamina it is apparent that the photosynthesis of the new lamina falls off rapidly with increasing light intensity. No explanation can be given for this apparent inhibition at high intensity. The electron micrograph of shallow lamina material (see Plate IV) did show a massive accumulation of polyphenolic material which may indicate that light may sometimes cause damage to Laminaria cells. The interesting feature of the data for new lamina material is that the efficiency of utilisation of low light (5% of full light: 0.249 cal/cm^2/h) is greater for the new lamina than for the old lamina. This feature could be of significance in the seasonal growth of the new lamina.

The importance of temperature is indicated in the increased compensation points obtained with the use of respiration rates at higher temperatures.

SUMMARY

1. The compensation points of the old lamina (shallow plants -0.45 cal/cm^2/h; deep plants -0.35 cal/cm^2/h at 5°C) and of the new lamina of shallow plants (0.498 cal/cm^2/h) have been calculated.
2. The old lamina has a saturation point of 1.66 cal/cm^2/h and the new lamina has a saturation point of 0.498 cal/cm^2/h.
3. Compensation points were increased with an increase in temperature.
4. Deep plants did not seem to be more efficient at using low irradiances compared to shallow plants.
5. New lamina tissue appeared to be slightly more efficient at using low light irradiances than old lamina material.
6. The reduced compensation point in deep growing plants was largely the result of a reduced biomass and hence a smaller respiratory loss than shallow plants.

Note. It must be emphasised that the results of the above experiments must be extrapolated with caution to conditions in the natural environment since the spectral quality of the lamp used was markedly different to the spectral distribution of irradiance underwater. The tungsten-halogen lamp used had a maximum emission in longer wavelengths (700-750nm) whereas the maximum transmission of underwater light is in the 500-550nm region.

CHAPTER 7

Respiration and the utilization of endogenous and exogenous reserves

Introduction

There have been numerous studies carried out on the respiration and metabolism of reserve carbohydrates in the Phaeophyceae, but much of this work has been confined to intertidal algae. Thus, Newell & Pye (1968) found that the respiration to temperature (RT) curve of several intertidal algae was modifiable in such a way that the shallow part of the curve is appropriate to the ambient temperature, thus minimising the effect of fluctuating temperatures on respiration. Kanwisher (1966) found some evidence of acclimatisation in several intertidal species, and also suggested that Arctic algae show a dormancy, with the winter respiration of Laminaria about half the summer rates.

Gail (1922) found that respiration rates of sublittoral algae did not change with depth. Tikhovskaya (1940) found that the respiration rate of L. saccharina was low in March and April, coinciding with the lowest temperatures. The Harder-Kniep theory (see Gessner, 1955) first advanced by Kniep (1915-1916) and later substantiated by Ehrke (1931), suggested that the respiratory rate of many cold water algae is held low during the spring and the ratio of photosynthesis to respiration is at its highest at this time, allowing the rapid growth observed. Milthorpe (1949) found the R.Q. of L. digitata was 0.75 and Clendenning & Sargent (1957, 1958) measured respiration in Macrocystis. Kain (1964) and Hopkin & Kain (1971) have measured the rates of respiration of zoospores and tissue discs of L. hyperborea.

The nature of the respiratory substrate and the physiological role of carbohydrates in the Phaeophyceae, and Laminaria in particular, has been investigated by several workers. Interest in the carbohydrates of brown algae from the commercial utilisation aspect led to a great deal of quantitative work on the seasonal fluctuations of the major carbohydrates in species such as L. hyperborea. Black (1950a) summarised the findings from 1946 to 1948. The features were that mannitol (a polyhydroxy alcohol), laminarin (a β-1:3 linked

glucose polymer), and the dry weight followed a similar seasonal pattern showing minimum in the spring and maxima in the autumn. The most marked variations occurred in the lamina with only small variations in the stipe.

Black's data is summarised in Table 7:1:-

Table 7:1

Seasonal variation in lamina mannitol and laminarin content as % dry weight after Black, 1950a,b).

Cullipool				Orkneys	
Mannitol		Laminarin		Mannitol	
March	6.4	May	1.0	March	8
August	22.9	September	32.0	August	38

Black (1950b) found that mannitol content increased with depth, reaching a maximum at 6-10m and decreasing below that, and he correlated the maximum mannitol content with maximum photosynthesis. Black (1948) had reviewed the earlier work which suggested that both mannitol and laminarin are storage compounds. Kylin (1915) first suggested that laminarin was a reserve carbohydrate and Nisizawa (1940) suggested that both mannitol and laminarin are storage substances. Black (1948) showed that laminarin did not appear in the lamina until the mannitol reached a definite concentration and suggested it was formed as a secondary product of photosynthesis in an analogous way to the formation of starch from glucose in higher plants.

Bidwell (1958a) showed that 85% of the photosynthetically fixed carbon 14 accumulated in the ethanol soluble fraction was in mannitol in L. agardhii. Yamaguchi et al (1966) showed that radioactivity was rapidly incorporated into mannitol during photosynthesis by Eisenia bicyclis and Bidwell (1967) confirmed that mannitol was the primary product of photosynthesis in brown algae. There has been less definite evidence about the metabolism of mannitol, largely due to the maintenance of a large endogenous pool of mannitol which has obscured changes in mannitol concentration. Thus Milthorpe (1949) found no significant change in the concentration of either mannitol or laminarin during dark starvation but pointed out that this was not proof that

they were not being used in respiration. Bidwell and Ghosh (1962) found that only small amounts of externally supplied C14-mannitol were metabolised by Fucus vesiculosis, but Drew (1969a) showed that most of the 'apparent' uptake of exogenously supplied mannitol was, in fact extracellular, and elutable, and the small amount of metabolism was put down to either a slow transport system or a preferential utilization of endogenous pools of mannitol.

Vaughan, quoted in Parker (1966) found that mannitol was more rapidly consumed during dark periods than other carbohydrates in Macrocystis and Yamaguchi et al (1966) showed that subsequent dark incubation of tissues, after assimilation of sodium bicarbonate-C14 resulted in a rapid decrease in the radioactivity and amount of mannitol, with the specific radioactivity of mannitol changing much more rapidly than the concentration. Bidwell (1967), in similar experiments, has reexamined the status of mannitol and came to the conclusion that it is the primary substrate of respiration.

The role of laminarin and the relationship between mannitol and laminarin is still not clear. Yamaguchi et al (1966) found that the specific activity of the glucose residues from the laminarin fraction decreased rapidly during dark incubation whilst an increase in activity in fucose and xylose occurred. They postulated that laminarin was a reserve substance and was being broken down in the dark with a concomitant synthesis of other carbohydrates taking place. It was further suggested that mannitol and laminarin were interconvertable in the same way as sucrose and starch in higher plants. Bidwell (1967), however, has refuted these findings and found no increase in the specific activity of complex compounds in sufficient quantities in dark periods for them to be considered analagous to starch in higher plants. Aspects of respiration and carbohydrate metabolism in L. hyperborea have been investigated in the present study.

1. Respiration studies

Respiration of tissues at various temperatures

Manometric studies were carried out on various tissues of L.hyperborea at different temperatures to obtain a rate-temperature (RT) curve from which

values of respiration for the various temperatures at which 'in situ' experiments were carried out could be interpolated. The Q_{O_2} was found from Warburg respirometry. Initial readings were often not used as considerable fluctuations were sometimes apparent due to non-equilibration and possibly iodide oxidation (Shaw, 1960). The respiratory rates, in µl O_2/mg dry wt/h. were converted to µgC lost/mg dry wt./h. using the R.Q. of 0.92, assuming that the respiratory substrate was mannitol. Evidence that mannitol is likely to be the respiratory substrate is given in a later section. Carbon losses are given in Table 7:2 for the various temperatures examined and the data is also shown in Figure 7:1.

Table 7:2

Respiration rates for tissues of L. hyperborea at various temperatures

Temperature °C	carbon loss µg C/mg dry wt./h Lamina	Stipe	Holdfast (Haptera)
4	0.31±0.06		
5		0.12	0.07
6	0.44		
10	0.51	0.14	0.06
12	0.47±0.08		
16	0.35±0.08		
20	0.28±0.02	0.10	0.17

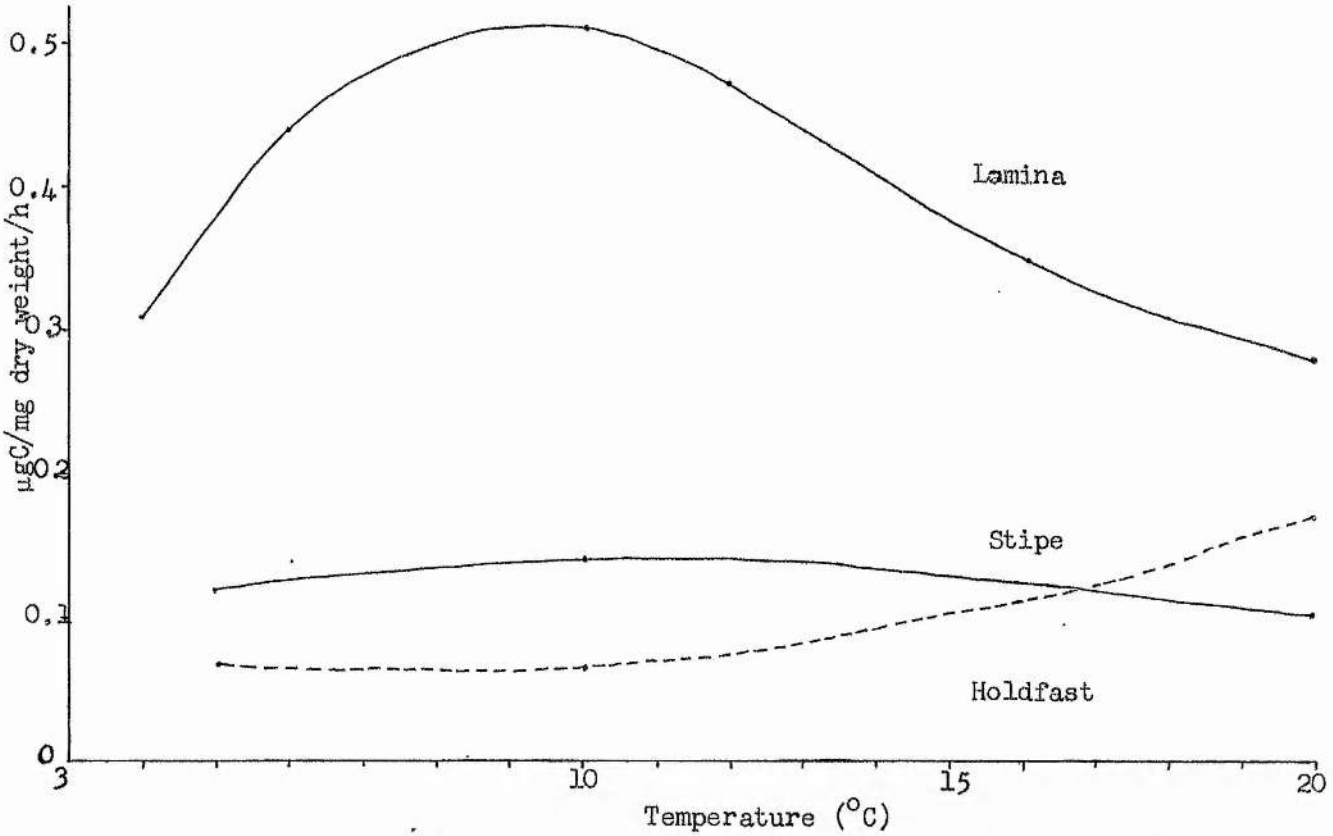


Figure 7:1 Respiration:temperature curve (RT) for tissues of L. hyperborea

The rates of carbon loss for lamina and stipe tissue were read off the RT curve at the appropriate temperature and used to correct 'in situ' photosynthesis measurements to daily net rates. It was not found possible to obtain satisfactory respiration data for the holdfasts on an area basis so these tissues could not be corrected for carbon loss.

Respiration at two depths

Shade-tolerant plants exhibit a low metabolism and have a lower respiration rate than shade-intolerant species (Grime, 1966). Spence & Chrystal (1970a,b) found reduced respiration rates in deep-growing shade adapted species of freshwater submerged macrophytes. The possibility that a similar adaptation existed in deep growing plants of L. hyperborea was investigated.

Experimental

The respiration rate of tissues of L. hyperborea plants from 3.1m and and 10.7m was measured at 7°C, the ambient sea temperature at the time of the experiment (20.1.71).

Results

The respiration rate as carbon loss for the tissues at from 3.1m and 10.7m are presented in Table 7:3.

Table 7:3

Respiration rates of shallow and deep plants

Depth (m)	Tissue	carbon loss µgC/mg/h
3.1	Lamina	0.38
	Stipe	0.16
	Haptera	0.11
10.7	Lamina	0.10
	Stipe	0.16
	Haptera	0.07

The data suggests that deeper growing plants of L. hyperborea have a lower respiration rate than shallow growing plants; however, the data is from only

one experiment and the value given for the 3.1m lamina tissue is considerably lower than the value at 7°C on the RT curve (Figure 7:1). Corrections for the respiration of deep growing plants were made from the RT curve.

2. The fixation of $NaHCO_3$-C14 and subsequent dark starvation of lamina discs of L. hyperborea

This experiment was essentially similar to those of Yamaguchi et al. (1966) and Bidwell (1967) but with no dark/light cycles, after the preliminary photosynthesis.

Experimental

Discs of 6.1 cm^2 area were cut from the basal parts of the lamina of one plant collected from 6.1m at Fife Ness. A previous experiment which had used several plants, indicated that there was considerable variation with tissue from different aged plants so only one plant was used. The discs were incubated in 5ml seawater with 1 μCi of sodium bicarbonate-C14 contained in small beakers which were clipped into a tray in the metabolic shaker bath. Photosynthesis was at 4.98 cal/cm^2/h and 8°C for 3.5 hours.

After photosynthesis the discs were removed, briefly washed in running seawater to remove residual C14-bicarbonate and placed in submerged plastic mesh cylinders contained in a box with running seawater, flushing through the system. Figure 7:2 shows the arrangement for dark incubation. The whole box was wrapped in foil to exclude all light.

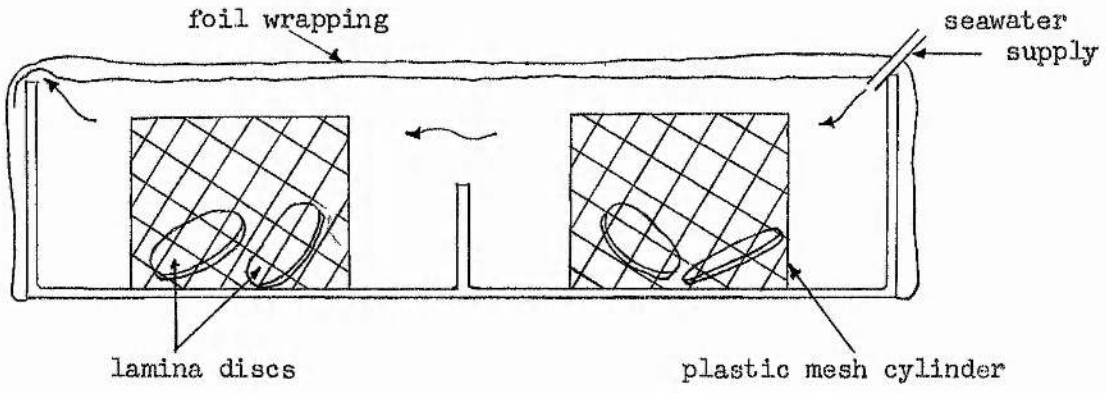


Figure 7:2 Dark incubation apparatus.

Two lamina discs were removed at zero time and killed in hot 80% ethanol. Samples were removed at intervals over the next 7 days.

The discs were further extracted, hydrolysed, and counted in the normal way.

GLC analysis

The carbohydrates in the ethanol soluble and acid hydrolysates were analysed using GLC. The ethanol extracts were prepared in the normal way as described in Chapter 2. The acid hydrolysates were neutralised, as for counting, and aliquots were taken from the neutralised hydrolysate and dried down 'in vacuo'. This extract was sylylated in the usual way.

It was assumed that the normal acid hydrolysis - using 1 N H_2SO_4 at 100°C for 3 hours - completely broke down all the laminarin into its constituent monomeric sugars, β-D-glucose and D-mannitol.

Results

Redistribution of Cl_4 radioactivity amongst the various fractions during dark incubation

The changes in the radioactivity of the fractions during dark

incubation are shown in Table 7:4. Two discs were analysed at each time interval and the pattern of redistribution of radioactivity, expressed as the percentage of total activity found in each fraction is shown in Figure 7:3.

Table 7:4

The redistribution of radioactivity in the various fractions during dark incubation

The total radioactivity (cpm) is given and the specific activity (SA) in cpm per mg dry weight.

Incubation time in dark	Ethanol soluble fraction total	S.A.	Acid hydrolysate total	S.A.	Residue Total	S.A.	Extr. dry wt. (mg)
0	43375	648	2216	33	-	-	67
9h	41058	496	3118	38	5027	61	82.8
18h	36195	475	3374	44	4119	54	76.2
1	37675	464	4436	55	4514	56	81.2
2	32100	518	4875	79	3389	55	62
3	32253	503	6122	96	5153	80	64.1
4	29063	454	4884	76	4385	69	64
5	22375	402	6096	110	4946	89	55.6
6	24150	294	5247	64	4700	57	82.1
7	15375	308	4015	80	2020	40	50

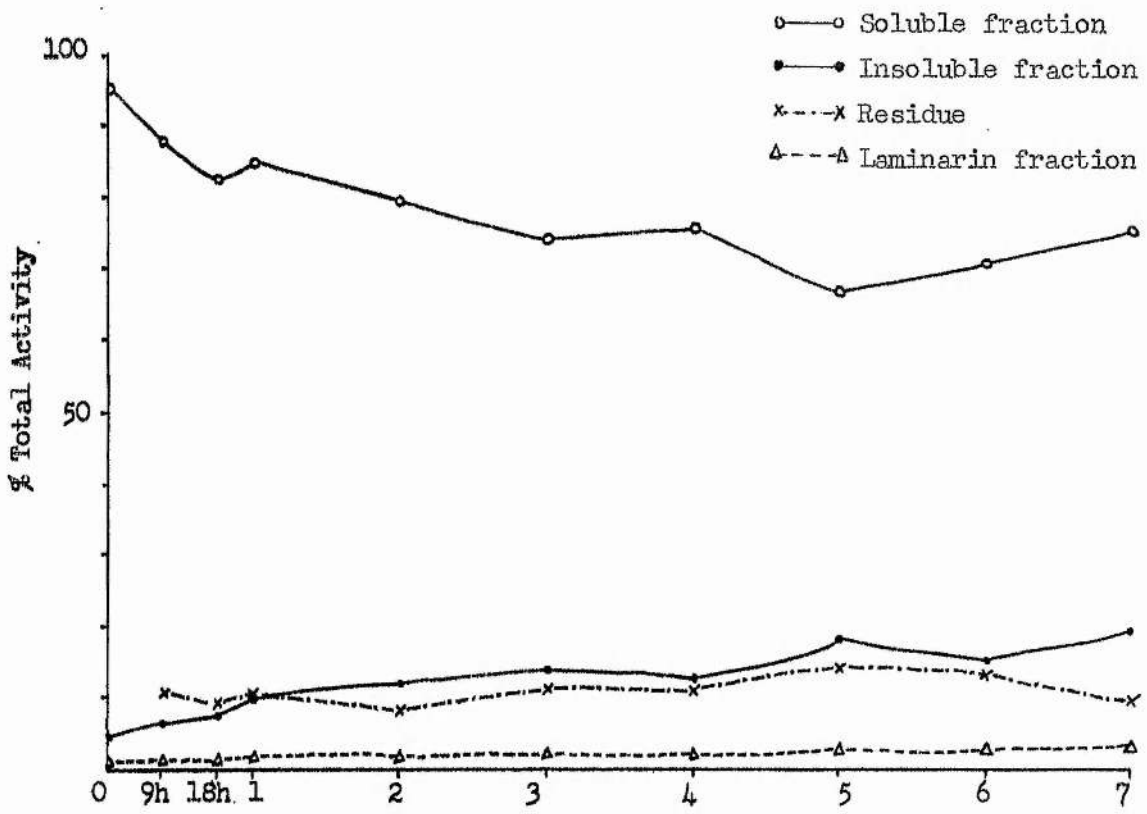


Figure 7:3 Distribution of radioactivity amongst various fraction during dark incubation.

The distribution of C^{14} radioactivity in the various fractions after the initial $3\frac{1}{2}$ hours incubation with C^{14}-bicarbonate in the light showed the alcohol soluble fraction contained some 95% of the total radioactivity. The acid hydrolysate contained the remainder as there was no detectable radioactivity in the residue. It can be seen from Table 7:4 that the activity in the ethanol soluble fraction changed significantly during the dark incubation. Thus, the total radioactivity after 7 days dark starvation was some 35% of the initial radioactivity in this fraction whilst the specific activity of this fraction was some 48% of the initial.

The total and specific radioactivity of the acid hydrolysate showed an increase of 40-55% after 7 days dark incubation compared with the initial values. The residue showed little change over this period.

It seems likely that one or more compounds in the ethanol soluble fraction were undergoing active metabolism in the dark and that the carbon 14 was being redistributed into compounds in the acid hydrolysate. No analysis was made of the radioactive compounds in each fraction but from the work of Bidwell (1958) it is very likely that C^{14}-mannitol was the main labelled assimilate in the ethanol soluble fraction as this author found that 85% and 93% of the soluble fraction radioactivity was found in mannitol in L. agardhii and L. digitata respectively after photosynthesis in C^{14} labelled seawater. Yamaguchi et al (loc cit) found that 85-90% of the total radioactive carbon incorporated into Eisenia after 3 hours photosynthesis was in the alcohol soluble fraction and that 60-70% of this activity was found in mannitol. Some indication that the metabolised compound in the soluble fraction was mainly mannitol is given in Figure 7:4 which shows the concentration of mannitol in the ethanol extracts over the same dark starvation period. Also shown is the concentration of glucose in the acid hydrolysates at each sampling time during dark incubation.

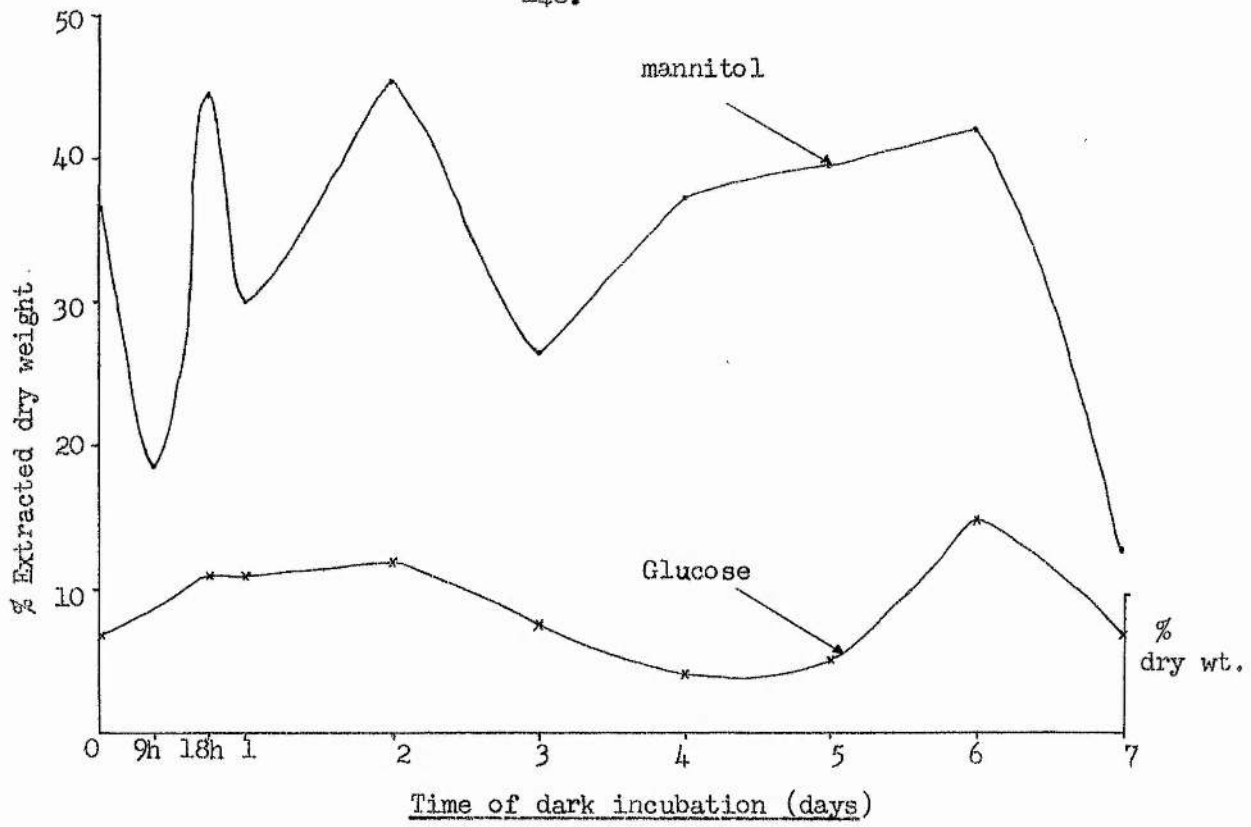


Figure 7:4 Changes in mannitol and glucose concentrations during dark incubation.

It is apparent that the mannitol concentration fell rapidly in the first 9 hours but maintained a fairly constant level for most of the dark incubation, then falling rapidly after 6 days to a value about 35% of the initial concentration at 7 days. Glucose tended to stay constant but reached a peak after 6 days dark starvation.

The changes in the concentration of mannitol were not as dramatic as the steady fall in the radioactivity of the ethanol soluble fraction, which is assumed to contain mainly C^{14} labelled mannitol, but the final value after 7 days dark incubation was about 35% of the initial in both cases.

Thus it may be assumed that the C^{14} labelled compound undergoing metabolism in the soluble fraction was mannitol which, in agreement with other findings, is held to be the primary substrate of respiration in L. hyperborea. The situation is less clear for the compounds in the acid hydrolysate. It appears that both the total and specific radioactivities of this fraction increase with dark incubation to reach a peak after 6 days. Since the acid hydrolysate represented the polysaccharides such as laminarin and fucoidin it

is apparent that these may be built up at the expense of the soluble compounds. The specific activities of the mannitol and glucose in these fractions have been estimated in the following way:

Changes in the specific activity of mannitol and glucose

In the absence of direct analysis of the specific activity of these compounds during dark incubation an estimate of them was made from a combination of the data and factors for the content of activity in these compounds from the literature.

Specific activity of mannitol

The specific activity of mannitol in each sample was estimated using a factor from Bidwell (1958a); in this paper mannitol was estimated to contain 85% and 93% of the soluble activity after 4-6 hours photosynthesis in C^{14} labelled medium in L. agardhii and L. digitata respectively. The average of these values or 89% was used as a factor to convert the total cpm in the ethanol soluble fraction found in each sample to a value for the radioactivity in mannitol. The mannitol content in µg was known from GLC analysis of each sample so the specific activity of the mannitol during dark starvation could be estimated in cpm/µg.

Similarly the activity in glucose was estimated from a factor obtained from the data of Yamaguchi et al (1966). These authors found that after 3 hours photosynthesis in C^{14} labelled medium, some 16% of the total radioactivity in the polysaccharide fraction was in laminarin. Thus, in a crude way, the acid hydrolysate found in this experiment was regarded as total polysaccharide and the fraction of this as laminarin was found using the factor of 16%. Since laminarin consists mainly of glucose with only some 2% as mannitol (Lewis & Smith, 1967), the specific activity of glucose was estimated using the total counts for glucose (i.e. equivalent to the value for laminarin) in each sample divided by the weight of glucose found from GLC.

Results

The data is presented in Table 7:5 and Figure 7:5.

Table 7:5

Specific activities of mannitol and glucose during dark incubation (activity in mannitol and glucose estimated for one disc only; i.e. not from mean data in Table 7:4)

Sample	mannitol CPM	mannitol µg	SA cpm/µg	glucose CPM	glucose µg	S.A. cpm/µg
0	43165	3100	1.39	337	505	0.67
9h	34599	21800	1.59	-	-	-
18h	29036	32200	0.90	710	615	1.15
1	29148	26700	1.09	862	860	1.00
2	31907	32600	0.98	712	760	0.94
3	33486	19500	1.72	1014	490	2.07
4	29926	25100	1.19	583	242	2.4
5	18690	26900	0.69	1063	312	3.41
6	26789	22700	1.18	521	685	0.76
7	8455	6000	1.41	406	283	1.44

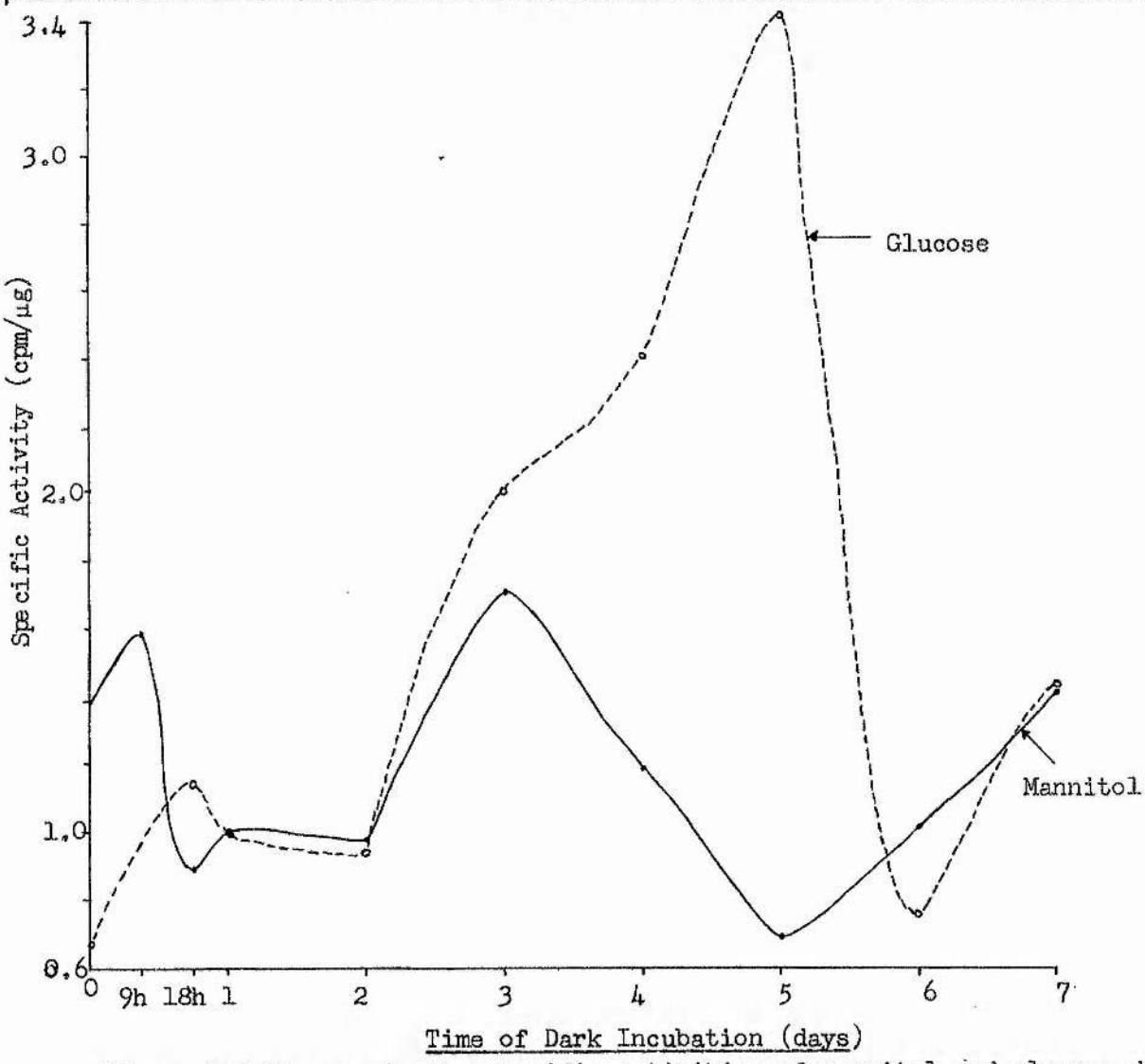


Figure 7:5 Changes in the specific activities of mannitol and glucose during dark incubation.

Conclusions

Mannitol

After an initial rise in specific activity there was a rapid fall and then a generally constant level apart from a peak at 3 days. The initial rapid fall in specific activity suggests a rapid metabolism of newly formed molecules. That rapid diffusion to mitochondria and metabolism of these molecules could occur is indicated in Plate III where the close proximity of a mitochondrion to the chloroplast would allow such movement. After this period there was, in general, little further metabolism of labelled mannitol and it appears that the mannitol may undergo some redistribution but is mainly stored in the vacuole. Black (1950a) mentions that mannitol will rapidly leach out of cells when placed in distilled water indicating it is probably in simple solution in the cell sap.

Glucose

The specific activity, of glucose, in general, showed a tendency to rise to a peak at 5 days, followed by a rapid fall. The specific activity of glucose did not decrease during the dark incubation as was found by Yamaguchi et al. (1966).

The relationship between mannitol and laminarin

It appears from Figure 7:5 that a reciprocal relationship between mannitol and laminarin does, to some extent, exist. Thus in the period of dark incubation from 9 hours to 18 hours the specific activity of mannitol falls whilst the specific activity of glucose shows a concomitant rise and from 3 days to 5 days a similar inverse correlation is evident between the two specific activities, with mannitol showing a minimum value at 5 days starvation when glucose shows a maximum value.

It is suggested therefore, that there is a relationship between mannitol and laminarin which is similar to the interconversion between glucose and starch in higher plants. The drop in mannitol is, however, not sufficiently great to account for the rise in glucose specific activity so it is possible that interconversions occur from other compounds such as fucoidin.

3. Heterotrophic uptake and metabolism of exogenous glucose by L. hyperborea.

Wilce (1967) put forward the hypothesis that Arctic deep-growing attached marine algae may have to rely on the heterotrophic uptake of organic compounds in poor light conditions found under ice and during the polar night. L. hyperborea produces a new lamina in January and February when, as a C14 'in situ' experiment showed (see experiment K, Chapter 5), no positive net photosynthesis occurs. It was, thus, pertinent to examine whether L. hyperborea could absorb exogenous organic compounds which might aid this growth under sub-compensation conditions.

Bidwell & Ghosh (1962, 1963) found that considerable amounts of exogenously supplied C14-glucose and mannitol were taken up by F.vesiculosis. The absorbed sugars were not metabolised to any degree and C14-mannitol remained as such in the tissues, whilst C14-glucose was not converted into metabolically useful mannitol. Drew (1969a) found that the C14-glucose supplied to 10 genera of marine macrophytes was, apart from Ascophyllum and Pelvetia, almost totally in the 'free space' of the alga and was readily eluted with washing. In these experiments L. digitata and L. saccharina were found to be poor heterotrophs of carbohydrate substrates and from 57-70% of initially accumulated C14, supplied as C14-glucose was elutable. The radioactivity in the soluble fraction was found to be exclusively in glucose indicating little metabolism and conversion to mannitol.

However, in the case of L. hyperborea the initial growth of the new lamina takes place in very poor light conditions in the sublittoral region (Kain, 1971) and it was conceivable that heterotrophic supplement to translocation might be significant.

Experimental

2cm by 0.5cm strips of epiphyte free tissue of the new and old lamina were cut from 3-6 year old plants of L. hyperborea. 2cm long sections were cut from the stipes and haptera of several plants.

15 lamina sections and 6 stipe and haptera sections were floated in 5ml filtered seawater contained in the main chamber of Warburg flasks. 0.2ml

of C^{14}-glucose (2μCi) was added to this medium and 0.3ml of 10% KOH was added to the centre well to capture CO_2. The flasks were wrapped in aluminium foil and secured to a tray in a metabolic shaker bath. Incubation was carried out for 4.5 hours in the dark at 9°C.

After incubation the tissues were briefly washed in seawater and transferred to flasks containing 5ml of fresh seawater and KOH which were foil wrapped and placed in a metabolic shaker bath at 1°C. for a one hour initial washing period. After this period the washing medium was removed and replaced with a further 5ml fresh seawater. The KOH was kept in the centre well for the second 2 hour washing period. After this final washing the tissue was removed, briefly rinsed in distilled water and killed in hot 80% ethanol. The tissue was fully extracted and counted in the usual way. The final medium, washing media, and the KOH were counted as described in Chapter 2.

The precaution of washing at near freezing and a change of the washing medium was to ensure that any eluted material was not re-absorbed from the medium. Significant heterotrophic utilisation of this organic substrate would be indicated by considerable amounts of 14_{CO_2} captued by the KOH during incubation and washing and also if the C^{14}-glucose was taken up into the soluble fraction and converted into metabolically useful mannitol.

Results

The results are given in Tables 7:6 and 7:7.

Table 7:6

Uptake of radioactive glucose by tissues of L.hyperborea (data in cpm)

Tissue	EtOH soluble	*EtOH insoluble	Total C^{14} in tissues	Total C^{14} in washings	Total initial C^{14}	% total washed out
OL	1645	824	2469	28550	31019	92
NL	555	22	577	31400	31977	98
S	12983	737	14629	27625	42254	65
H	4345	1186	9583	10325	19908	52

* combined acid hydrolysate and residue.

Table 7:7

Uptake of Cl4-glucose by tissue sections and respiration of labelled carbon (data in cpm/mg dry weight)

Tissue	EtOH soluble	EtOH insoluble	Insoluble as % of retained Cl4	*Total CO_2	Specific activity of CO_2 (cpm/mg dry wt/µgC respired)
OL	48	24	33	9.8	0.13
NL	25	1	4	4.5	0.09
S	125	16	11	2.9	0.04
H	73	87	55	7.4	0.42

* total $^{14}CO_2$ captured during incubation and washings (7.5 hours)

The data in Tables 7:6 and 7:7 indicate that L. hyperborea does not retain much of the exogenously supplied glucose and the amounts washed out from old lamina and new lamina tissue are greater than the amounts washed out of L. saccharina and L. digitata lamina discs by Drew (1969a). The stipe and haptera tissue did show a higher degree of retention of the labelled sugar than the lamina tissue. Jackson (1971) also found regional variability in the uptake of Cl4-glucose by F. vesiculosis.

Of the radioactivity retained by the tissues some conversion to insoluble compounds had occurred and the highest value of retention and conversion to insoluble compounds was found to be in the haptera tissue. That some metabolism of the labelled substrate was occurring is indicated by the capture of $^{14}CO_2$ during incubation and washing.

However, it is clear that the radioactivity retained and metabolised by the tissue is low and most of the Cl4 taken up was leachable. The specific activity of respired carbon was low compared with data obtained by Jackson (1971) where values of 5-9.0 cpm/mg dry wt/µg C respired were obtained for Pelvetia, which was shown by Drew (1969a) and Jackson (loc cit) to be a reasonably efficient heterotroph and to be capable of producing mannitol from glucose.

Since it has been shown that mannitol is the primary product of

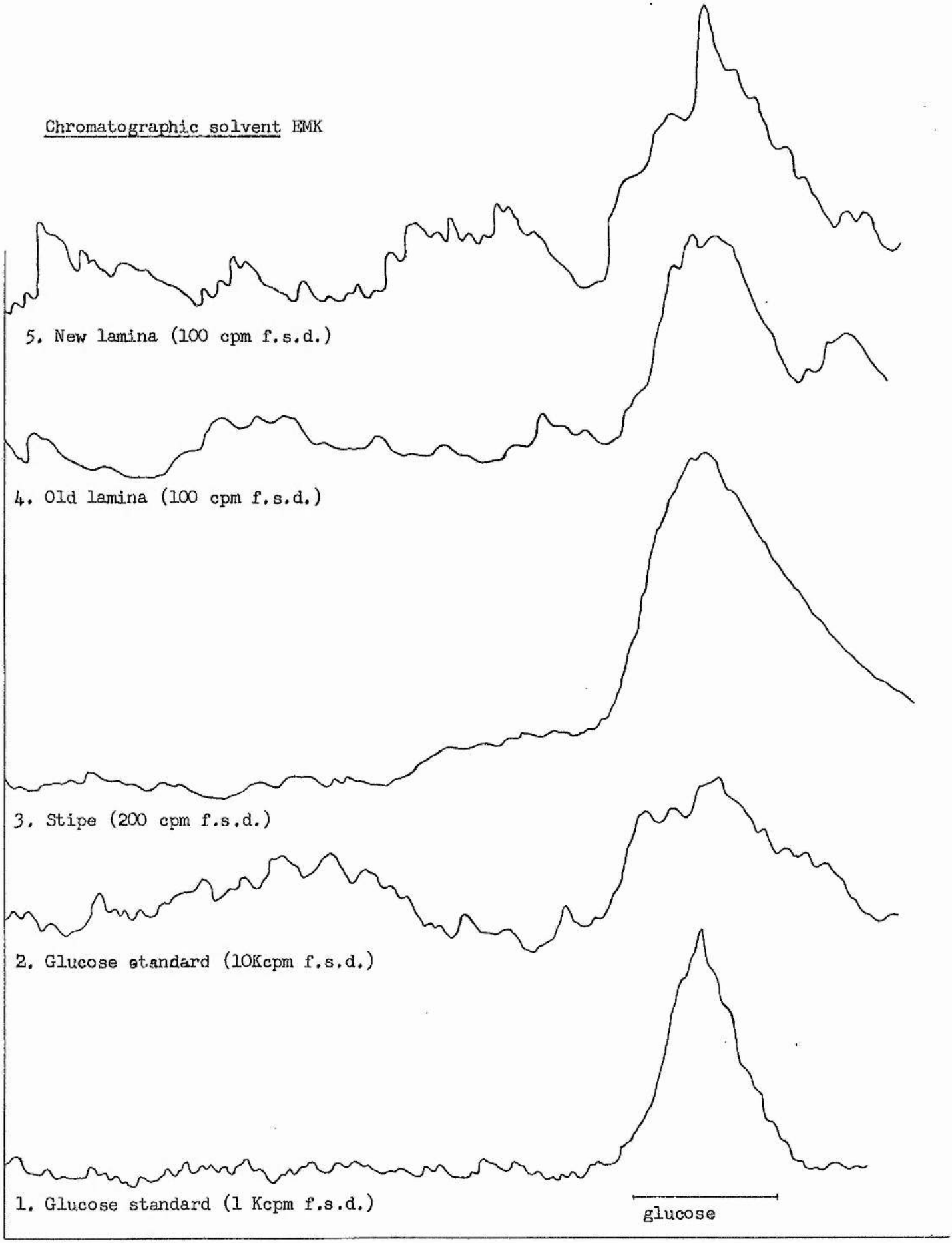


Figure 7:6 Distribution of radioactivity in the alcohol soluble fraction of tissues of L hyperborea after heterotrophic uptake of C14 glucose

photosynthesis and also a major metabolic substrate in Laminaria the uptake of organic substrates by the alga would only be of metabolic significance if the glucose substrate was converted to mannitol.

Aliquots of the alcohol extracts of each tissue were spotted on Whatman No 1 paper and run in the E.M.K. solvent. The individual strips for each extract were run in radiochromatogram strip scanners and the resulting labelled compounds identified. In all tissues the radioactivity in the soluble extract was confined to glucose as indicated in Figure 7:6.

The results for the uptake of C14-glucose by L. hyperborea show that it is an inefficient heterotroph and does not convert retained glucose into mannitol. The plant shows similar features in its heterotrophic metabolism to F. vesiculosis as shown by Jackson (1971) who concluded that most of retained glucose was bound to cell wall material. In all, it seems that heterotrophic uptake of organic compounds is an insignificant process for the accretion of carbon, and growth under very low light conditions must rely largely on reserve compounds.

4. Seasonal and depth variations in the soluble carbohydrate content of lamina, stipe and holdfast tissue

The seasonal pattern of the mannitol content of the lamina and stipe of L. hyperborea has been extensively investigated by Black (1948, 1950a,b) but, in view of the possible involvement of mannitol in translocation, a more detailed study was carried out to analyse the contents of mannitol in the old lamina, new lamina, and the outer and middle stipe, the outer and middle holdfast during the lag and rapid growth phases from January to June. This was carried out to see if seasonal gradients of mannitol concentration could be observed in the growing tissues of the new lamina, stipe, and holdfast, in a similar way to the seasonal gradients observed in the phloem tissue of white ash by Zimmerman (1957).

Plants were collected from Arisaig or Fife Ness and sections of old lamina, new lamina if present, stipe and holdfast, were killed in 80% ethanol,

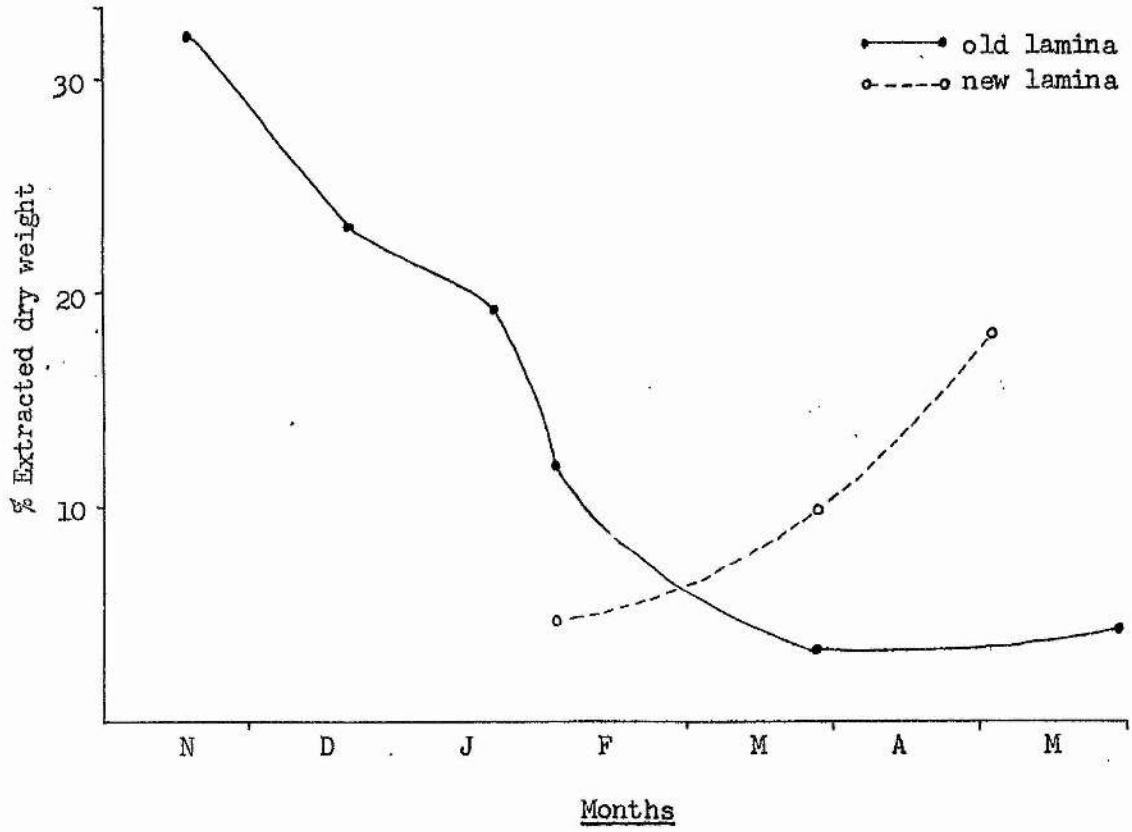


Figure 7:7 Seasonal variation in mannitol content of old and new lamina

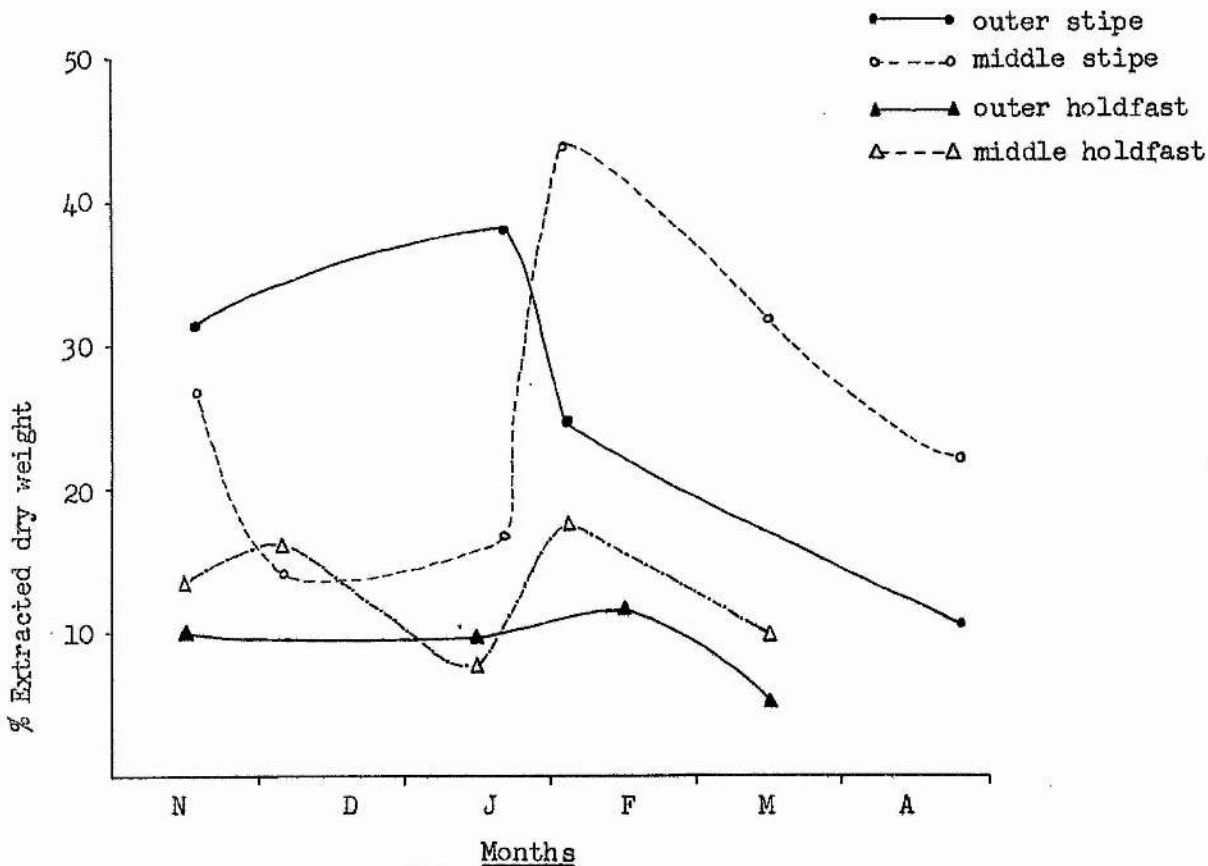


Figure 7:8 Seasonal variation in mannitol content of stipe and holdfast tissue

The stipe sections were taken from the upper part of the stipe near the transition zone and were divided into the middle stipe, including the medulla and a few cortical cells, and the outer stipe which consisted of the remainder of the cortex and meristoderm. The middle holdfast consisted of the mid-core of tissue and the outer holdfast was made up of haptera segments.

The plant tissue was completely extracted with 4 changes of ethanol and aliquots analysed for mannitol content with the GLC.

Results

The seasonal variation from November to May of the tissues is shown in Figs. 7:7 and 7:8.

Lamina Tissue

A fall in the mannitol content of the old lamina from November to March observed in Figure 7:7 was also found by Black (1950a). The new lamina in February was found to contain 4.6% (dry weight) of mannitol, and, as has been shown in chapter 5, the new lamina is below its photosynthetic compensation point at this time. The mannitol content indicated here was probably translocated into the new lamina from the old lamina. Once photosynthesis starts in March the mannitol content of the new lamina increases. Black (loc cit) found a mannitol content of 4% in the old lamina and 8% in the new lamina in April.

The rapid increase in mannitol content takes place after the time when photosynthesis increases in the new lamina. The mannitol content of the old lamina at this time remains low even though there is ample light for photosynthetic production of mannitol. The old lamina finally falls off in May or June.

Stipe tissue

The outer stipe tissue showed an increase in mannitol content from November to January and then a rapid decrease to April. The middle stipe tissue showed an initial fall in mannitol content but a rapid rise in mannitol content was found in January with a reduction during subsequent months.

It is suggested that the increase in mannitol content of the stipe tissue represents a concentration gradient which may arise in either the

remainder of the stipe or, more likely, from the old lamina.

This data can be correlated with the biomass changes observed in Fig.3:10 which showed that stipe growth starts in January and reaches a peak in March. The fall in mannitol in the stipe tissue seen in Figure 7:8 may be correlated with the utilisation of this respiratory substrate during the spring growth.

Holdfasts

The middle holdfast tissue does not show much variation over the period studied but there was a peak in February. Similarly, the outer holdfast tissue showed a peak in February which may indicate an accumulation prior to the growth in biomass seen in Figure 3:10. This growth may occur at the expense of this influx of mannitol, since a drop in mannitol content was seen from February to March.

Variation in mannitol content with depth

The ethanol extracts from samples in January were analysed for mannitol content using GLC. It was found that the mannitol content of all parts of the plant decreased with depth. The data is set out in Table 7:8.

Table 7:8

Concentration of mannitol (% dry wt) with depth (January)

Depth	Lamina	Outer stipe	Middle stipe	Outer holdfast	Middle Hfst
3.1	19.3	38.3	16.6	22.3	9.0
10.7	9.4	17.7	17.7	11.9	6.9

However, when the ethanol extracts for the C14 'in situ' experiment were analysed the situation was reversed. This was an experiment in July and the results are in Table 7:9

Table 7:9

Concentration of mannitol with depth (July)

Depth	% dry weight
3.1	31.1
10.7	32.4
18.3	52.2

Discussion

The respiration rate of lamina tissue of L. hyperborea was found to have an optimum temperature of 9.5°C and a further increase in temperature caused an inhibition of the respiratory rate. The data for this RT curve was obtained in November whilst an experiment in March indicated similar rates. This suggests that there is not a seasonal adaptation in this species of Laminaria.* Kanwisher (1966) found for an Arctic sublittoral species of Laminaria that the respiratory rate fell during the winter and this resulted in a smaller drain on reserves. However, these algae have to survive the polar night under a considerable snow and ice cover and L. hyperborea has to survive under poor light conditions only from January to March. The importance of low respiration rates at the low sea temperature during the rapid spring growth phase is emphasised and, this, coupled with the fact that rapid photosynthesis can occur under low light conditions may be the main reason for the rapid growth observed between March and June. The fall off in the growth rate in June is probably a reflection of the increased biomass by that time which reaches a limit when the photosynthetic tissue at the surface of the plant can no longer support the respiring tissue beneath it.

The critical importance of the interaction between photosynthesis and respiration can be seen in the gradual reduction in the upper limit of colonisation by various species of Laminaria from the English Channel to the Straits of Messina. In the Channel L. ochroleuca grows up to the surface and can compete successfully with L. hyperborea (John, 1969) whereas in the Straits of Gibraltar this species is not found above 40m off the Island of Alboran (Pérès, 1967) and was dredged up from 30m off the Algerian coast by Feldman (1934). On passing further into the Mediterranean basin the upper limit of this species is at even greater depths. Fredj (pers. comm.) and Fredj & Giermann (1968) observed populations of L. ochroleuca, (some of the larger specimens with a lamina 5m in length) at 110m from the diving saucer SP300 in the Straits of Messina, Sicily. Fredj (1969) also found populations of L. rodriguezii at

* See Addendum, page 206 for a consideration of the recent data of Lüning (1971a).

Revellata, Corsica in a distinct zone between 75m and 90m. Pérès (1967) had commented on the 'euryphotic' nature of these species and the irradiance at these depths was reported as reasonable, whilst temperatures were 24°C on the surface, 12.7° at 85m, and 12.5° at 90m. Fredj (1969) commented that it seemed these deep growing algae were existing in a stable thermal zone. It would appear that these Laminaria species are distinctly steno-thermal and this bears out the low optimum temperature found for respiration in L. hyperborea. The higher temperature tolerance of S.polyschides(Kain, 1969) allows it to grow at the surface in the Straits of Messina (Drew, pers. comm.). It is possible that a rise in temperature from 10° to 20°C will cause some protoplasmic or enzyme damage in L. hyperborea.

It was indicated in section 1 that deep growing plants may have reduced respiration rates compared to shallow plants. Reduced attrition rates have been noted by Goreau (1963) and Drew (1969b) in calcareous red algae and this could be of great ecological significance for the growth of deep plants.

In section 2 it was indicated, in line with other data for brown algae, that mannitol is the primary substrate of respiration. It is likely that much of the mannitol synthesised in photosynthesis is stored (hence the often large concentrations found in these algae) or, as suggested here, may be redistributed to build up insoluble compounds. Quillet (1957) has suggested the steps from mannitol to laminarin via a preliminary oxidation to fructose and then an isomerisation to glucose which is then polymerised to laminarin. The data presented here is in agreement with that of Yamaguchi et al (1966) who suggested an interconversion occurs between mannitol and laminarin.

In section 3 it was considered that heterotrophy is insignificant, at least as far as the uptake of glucose is concerned, and the process is not likely to assist the growth of the alga. It is possible that the uptake of organic substrates in highly polluted waters may be the only way the alga may survive (John, 1968) but there is little evidence to suggest that heterotrophy could assist the rapid growth of the alga in the spring.

There was some indication that gradients of mannitol exist in the

plant which may be correlated with the seasonal growth of various parts of the plant.

The variation in mannitol content with depth of submergence was found to be correlated with season. Thus, in January, plants at 3.1m had a higher concentration of mannitol than plants at 10.7m. In July, however, the situation was reversed and plants at 18.3m had the maximum amount of mannitol. Black's (1950b) data suggest that the mannitol values for lamina tissue in the Orkneys is greater at 12m in the period June to August than either the values at 4 and 8m.

It is possible that in shallow water the greater biomass of lamina found there (in older plants at least) may deplete the reserves of mannitol in the summer. It was apparent from data in chapter 3 and that of Lüning (1970a) that the lamina at depth continues growing after the slowing of growth in shallow plants. It is possible that the still improving light conditions at depth in the summer allow a greater build up of mannitol at depth compared with shallow laminas.

CHAPTER 8

Pigment and Lamina Morphology changes with Depth and Light Intensity

The 'in situ' experiments of Gail (1922), Printz (1939) and Levring (1947) confirmed the view of Engelmann (1883) that spectral quality is important in controlling the vertical distribution of the different coloured algae in northern coastal waters. However, the differences between the three algal types was not great and Harder (1923) had put forward the important view that both light quality and light intensity are important in any considerations of vertical distributions of algae. Adaptations to low light intensities in higher plants include increased pigment concentrations and the current recognition that both light intensity and light quality are important factors in controlling the depth distribution of a particular species has come from the work of Lubimenko (1908) and Strain (1950), who found that deep growing algae often had increased pigment concentrations and from the direct observations of Gilmartin (1960), Goreau (1963), and Larkum et al (1965,1967), who found that green algae may be found to considerable depths in clear tropical waters where the predominantly blue light can be utilised.

As regards one particular species, there has been little evidence to suggest that a marine alga has 'sun' and 'shade' adapted forms. It is known that the content of the various pigments can fluctuate depending on locality, depth, and season but there has been little work to suggest that such modifications are true light adaptations and aid absorption of light. It is possible that the increased pigments are a consequence of the lowered light regime and do not constitute a true light adaptation. Brody & Emerson (1959) found that low light intensity grown cells of Porphyridium had five times as much chlorophyll 'a' as high light grown cells. Terborgh & Thimann (1964) found an increase in the chlorophyll content of Acetabularia at low light intensities compared to high light grown plants.

In chapter 5 it was noted that deep growing plants of L. hyperborea have increased photosynthetic efficiencies and a thinner lamina than shallow growing plants. In this chapter the effect of light intensity on pigment concentration and lamina morphology has been investigated.

Results

1. Pigment concentrations at different depth and during the season

(a) Winter pigment concentrations at two depths

Plants were collected on 19.1.71 from 3.1m and 10.7m at Fife Ness. The lamina tissues were kept in dark conditions and 2g fresh weight sections were extracted the following day and analysed for pigment concentration as described in chapter 2. The data is given in Table 8:1.

Table 8:1

Winter lamina pigment concentrations at two depths (mg/g fresh weight)

Depth (m)	Total chlorophyll (ether)	90% acetone				CS_2
		chlorophyll a	chlorophyll c	total (a+c)	Ratio a:c	Fucoxanthin
3.1	0.150±0.01	0.115	0.039	0.154	2.95	0.07
10.7	0.200±0.01	0.139	0.031	0.170	4.48	0.08

The difference between the mean values for total chlorophyll at each depth was significant at P=0.05.

The data suggests that there was a 33% increase in the total chlorophyll content at 10.7m compared to the value at 3.1m. Chlorophyll 'c' and fucoxanthin content remain unchanged with depth and the change in chlorophyll was entirely due to an increase in chlorophyll 'a'. The value of chlorophyll 'a' at 10.7m was 21% greater than the value at 3.1m. This increase was also reflected in the increased chlorophyll 'a' : chlorophyll 'c' ratio at depth.

When, however, the data was based on lamina area (Table 8:2) the difference is no longer seen. This is due to the decreased weight per unit area depth.

Table 8:2

Chlorophyll content in mg/cm^2 lamina at two depths

Depth	Area of 2g.fwt. (cm^2)	Total chlorophyll (mg/cm^2)
3.1	15.3	0.02
10.7	20.7	0.07

Hellebust & Haug (1969) found values of between 0.029 and 0.034 mg chlorophyll 'a'/g fresh weight for meristoderm tissues of stipe sections of L. digitata. The stipe might be expected to have less pigment content than the lamina.

(b) Summer pigment concentrations at two depths

A further extraction was made of the old lamina and new lamina from 4.6mg on 16.6.70, and the lamina of plants from 3.1m and from 10.7m on 8.7.71. The data is given in Table 8:3.

Table 8:3

Summer lamina pigment concentrations at two depths. (mg/g fresh wt).

Depth (m)	Tissue	Total chlorophyll (ether)	90% acetone				CS_2
			chlorophyll (a)	chlorophyll (c)	Total (a+c)	Ratio (a:c)	Fuco-xanthin
4.6	Old lamina	0.421	0.340	0.335	0.675	1.0	0.187
	New lamina	0.525	0.376	0.384	0.760	0.98	0.220
3.1	New lamina	0.343	0.212	0.298	0.511	0.71	0.148
10.7	"	0.423	0.298	0.314	0.612	0.95	0.156

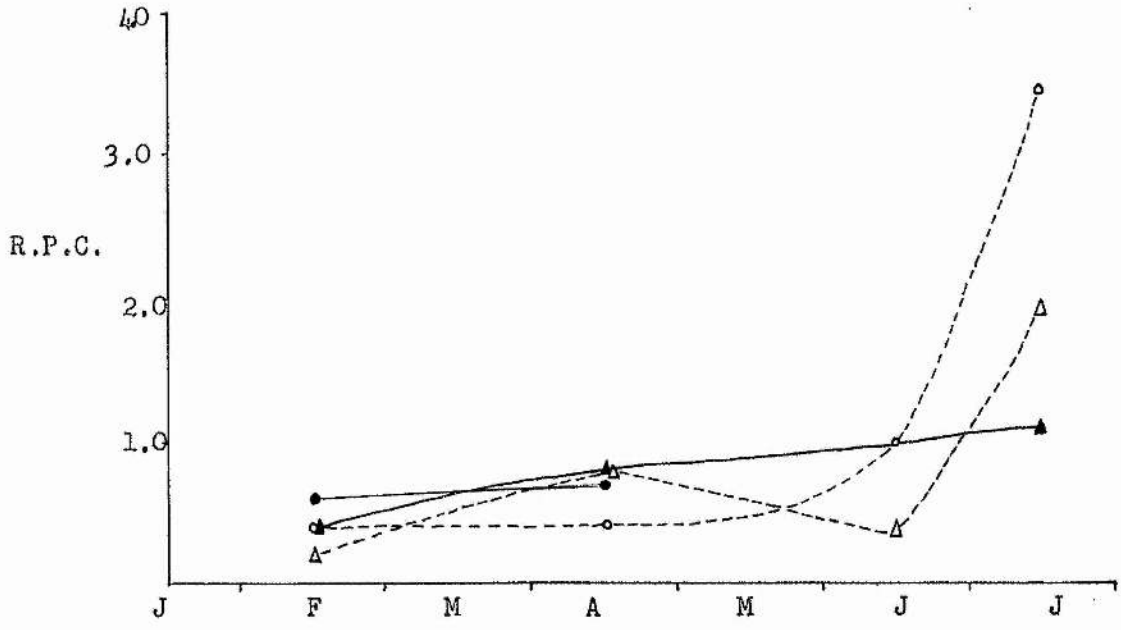
Table 8:4 Chlorophyll content in mg/cm^2 at two depths

Depth	Area of 2g fresh weight	Total chlorophyll (mg/cm^2)
3.1	19.3	0.036
10.7	22.1	0.039

Again, it can be seen that the chlorophyll content increased with depth but the increase of 24% of the value at 3.1m is slightly less than that found in January. This is possibly due to the increased light irradiance at 10.7m in the summer. There was again no change in the fucoxanthin content with depth.

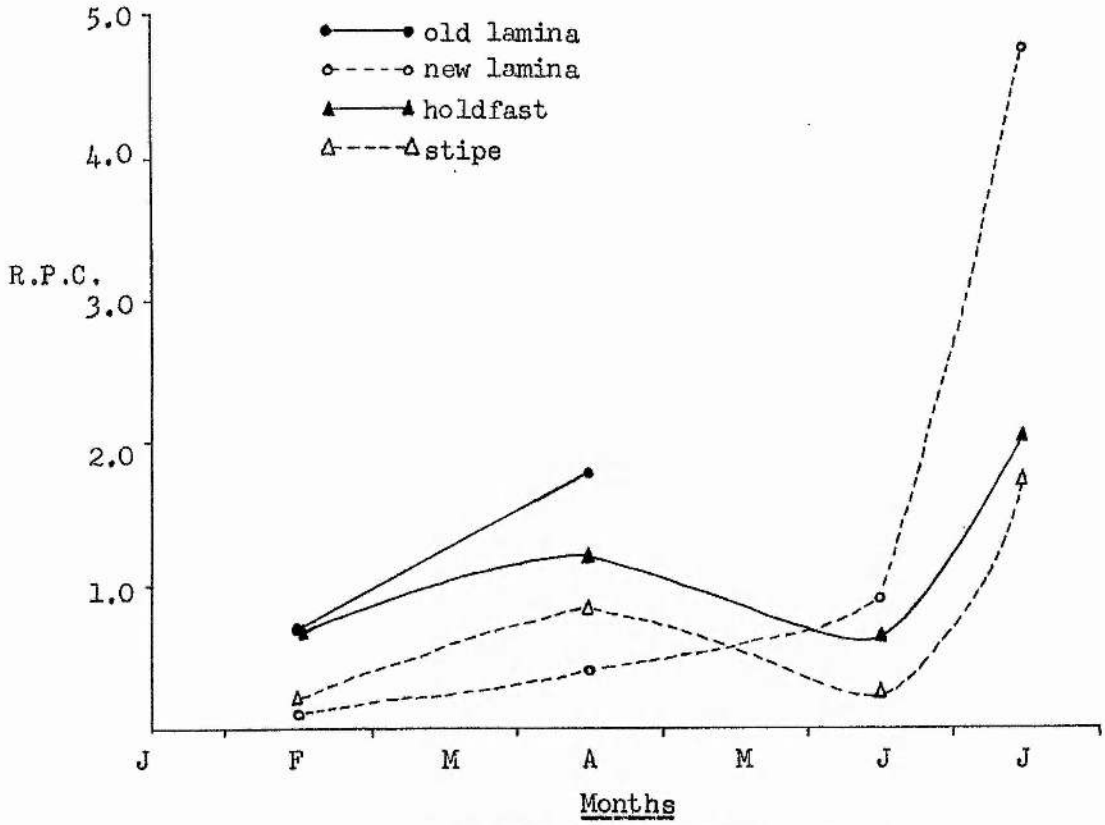
The old lamina analysed in June still contained a considerable amount of pigment. The summer values for pigment content were more than twice the values found in January (Table 8:1).

(a) Above canopy



Months

(b) Below canopy



Months

Figure 8:1 Seasonal variation of relative pigment component (RPC) above and below the forest canopy.

Seasonal variation in pigment content

An estimate of the seasonal change in pigment content of lamina, stipe, and holdfast tissue during the growing season from January to June was made using the 80% ethanol extracts from the various C14 'in situ' experiments carried out during this time.

Procedure

A known volume from each extract was transferred to a 1cm cuvette and the percentage absorption read over a number of wavelengths to determine the maximum absorption point. The value for percentage absorption at this wavelength was multiplied by a dilution factor for the complete extract volume and this gave the Relative Pigment Component (RPC). This factor represented the pigment content of each sample.

Note

Ethanol and especially aqueous ethanol, is not a standard solvent for pigments (methanol has been used by Steeman-Nielsen, 1961) but the extracts were used to give a relative analysis of pigment changes during the season. The data are shown in Figure 8:1 and it can be seen that pigment content was low from February to June when a sharp rise in pigment content was observed. It is interesting to note that the data for tissues below the canopy were often slightly higher than those of the tissues above the canopy. The old lamina had a high pigment content during this time and did not display any great loss in pigment as might be expected if it were a senescing organ (see Goodwin, 1965).

2. Lamina morphology under controlled light conditions in culture

In order to examine the possibility that light intensity is a controlling factor involved in the changes in lamina morphology noted in chapters 3 and 5, young sporophytes of L. hyperborea were maintained in culture under low and high light intensities with all other conditions similar.

The cultures were kept as described in chapter 2 at a high light intensity of 1.78 cal/cm^2/h* and the low light cultures were maintained at 0.22 cal/cm^2/h. Cultures were kept for 43 days at 10°C under these light regimes

* Although the high light intensity used may have been inhibitory, Kain (pers. comm.) considers this unlikely.

with gentle aeration in each culture.

At the end of this period the healthy plants were removed, blotted dry, and weighed. The plants ranged in size from 0.4-1.5cm in length (see Plate I). The area of each lamina was estimated by drawing around each plant onto paper and comparing the weight of this area of tracing paper with a known area. The results are shown in Table 8:5.

Table 8:5

Specific lamina area at high and low light intensities

Treatment	Number of plants	Fresh weight (mg)	Area (cm^2)	SLA (cm^2/mg fresh wt)
High light (1.78 cal/cm^2/h	55	67.2	4.1	0.061
Low light (0.22 cal/cm^2/h	91	80.0	7.7	0.096

The SLA in the low light grown plants was 57% greater than the value for the high light cultured plants.

Evans (1966), in a review of the chloroplast ultra-structure in several genera of the Phaeophyceae, showed that in all cases the thylakoids in each unit lamellation did not mutually cohere but ran in 3 parallel bands and only occasionally in 4 bands. However, Cole et al. (1968) reported an unusual feature found in cultured sporelings of Leathesia difformis but not in field material. Constant 3 thylakoids banding with no cross links was shown but, in many cells, extra bands of thylakoids were present between the normal continuous ones, the whole giving the appearance of an extensive stack of thylakoids. The light conditions in the cultures were suggested as the probable cause of extra thylakoid production and it is known that thylakoid formation occurs in response to light (Kirk & Tilney-Bassett, 1967). Working with the unicell Chlorella vannielii, Reger & Krauss (1970) found that low light (300 ft-c) cultured cells had 9.4 times the total chlorophyll on a dry weight basis than high light (6000 ft-c) cultured cells. Electron microscopy revealed similarities between chlorophyll

concentration and total lamellae, and large numbers of granum-like regions were present in the 300 ft-c culture whilst much fewer thylakoids were present in the 6000 ft-c culture.

Lamina tissue from shallow and deep growing plants and from high and low light cultured plants, grown at light intensities equivalent to those in shallow water and at the limit of the kelp, were used for electron microscopy to see if the changes in pigment content with depth were reflected in any morphological changes in the ultra structure of the chloroplasts, or more precisely, the chromatophores.

Thus young sporophytes were cultured at high light (1.78 cal/cm^2/h = 494 μg cal/cm^2/s) and low light (0.22 cal/cm^2/h = 61 μg cal/cm^2/s) corresponding to light saturation and light limiting conditions for the growth of early sporophytes (Kain, 1965). The cultures were maintained for 66 days under these conditions and then packaged in wrapped slide containers with some small discs of the lamina from shallow and deep growing plants. These were kept cold during transport to Dr. L.V. Evans, Department of Plant Sciences, University of Leeds who prepared the electron-micrographs shown in Plates II toV.

Results

It is apparent from Plates II to V that there is no alteration in the ultra-structure of the chloroplasts of either low light cultured sporophytes or deep growing plants compared to the structure of chloroplasts of high light grown and shallow plants.respectively. It should, however, be noted that the subepidermal cells of the shallow growing plants are full of a polyphenolic material (Evans, pers. comm.), a feature which may indicate a protective or wound response to high and possibly damaging light intensity. The increased chlorophyll concentrations reported in Section 1 may be due to either an increasing number of chloroplasts per cell or to an increase in the individual size of chloroplasts or to both. Rabinowitch (1956, Vol II, Part II, page 1259) stated that an increase in size of chloroplasts would be a less efficient way of increasing absorption of light than an increase in numbers. A crude estimate of chloroplast volumes in the cultured material was attempted, assuming that the E.M. sections

were cut through the median pole of the elliptical shaped chloroplast, but results were variable and no further attempt was made to examine the anatomical accomodation of the increased chlorophyll content. An increase or decrease of up to 50% of the volume of chloroplasts has been shown under varying light irradiances (Mayer, 1971).

DISCUSSION

Pigment contents for lamina tissue of L. hyperborea from 3.1m and 10.7m have been measured and there was found to be a 24-33% increase in total chlorophyll on a fresh weight basis in the deep growing plants compared to the shallow growing plants. It is not certain that such an increase in chlorophyll content could materially assist the deep growing alga to survive the much reduced light intensities at this depth (about 5% of that at the surface). The increase was not large. However, the increase occured under low light intensities and the values were in the range where absorption of light is proportional to chlorophyll concentration (i.e. 2 to 4 mg chlorophyll/dm^2) (Gabrielsen, 1948), and even a small increase such as this may have a significant effect on the ability of the alga to utilise incident light energy.

From chapter 6 it was noted that deep plants were slightly less efficient at utilising low light intensity (5% full light) than shallow growing plants although the differences were not great. In view of the low light levels the deep plant has to contend with, the maintenance of a high photosynthetic efficiency may be critical.

Steeman-Nielsen (1961) and Reger & Krauss (1970) also found that high and low light grown cells of Chlorella had much the same photosynthetic capacity at low light intensities. The latter authors found that the high light cultured cells, when corrected for dark respiration, were twice as efficient as the low light cultured plants at low light intensities. They also calculated that the low chlorophyll multiplied by the greater efficiency in the high light cells about equalled the low efficiency, but larger chlorophyll content, in the low light cells. This, in fact, is much the same as is found here. Thus the photosynthetic efficiency of the deep plant at 0.249 cal/cm/h was 5.22% and the photosynthetic efficiency of the shallow plant at the same irradiance was 6.0%. The increased efficiency in the shallow plant is balanced by the reduced amount

of chlorophyll as is shown in the calculation:-

	Photosynthetic efficiency (at 0.249 cal/cm^2/h)		Total chlorophyll (mg/g fresh weight)		
shallow plant	6	x	0.115	=	0.69
deep plant	5.22	x	0.139	=	0.73

It would thus appear that the increase in chlorophyll content at depth compensates to some extent for the slightly reduced efficiency, compared with shallow plants, of deep growing plants and it is possible that this increase in chlorophyll is of ecological significance to survival at depth. The high photosynthetic efficiency found at low light intensities in shallow plants confirms the view that these plants are 'euryphotoic' and can survive a wide range of light intensities.

The high content of pigment found in the oldlamina in June and the high photosynthetic capacity of this tissue found in an 'in situ' experiment in May (experiment N, Chapter 5), indicate that the old lamina does not undergo a great deal of senescence before it is removed. Hellebust & Haug (1969) noted the surprisingly high photosynthetic capacity of the old lamina in L. digitata but found that little photoassimilated C^{14} entered alginate indicating little active synthesis or growth. Thus a factor such as wave action is likely to be more important than senescence in the removal of the old lamina.

Studies on the seasonal changes in pigment content indicate that tissues

under the canopy exhibit the characteristics of deeper growing plants and have increased pigment content compared with the tissues of plants growing above the canopy. This increased pigment content could have some survival value.

The data on lamina morphology indicated that light may be a controlling factor in the development of the thinner lamina at depth. Thus the values of SLA obtained in culture differed as did the values obtained for older plants in shallow and deep water (see Figure 3:6chapter 3). There is, however, some evidence in the literature to suggest that lamina morphology is largely controlled by wave action. Thus, Svendsen & Kain (1971) have delimited the new form L. hyperborea f. cucullata to describe the phenotype of L. hyperborea found in very sheltered regions in Norway (see also Kain, 1971b). These growth forms have tortuous stipes tapering to a large, thin, brittle lamina and are restricted to habitats with little water movement. That turbulence and wave action could influence lamina morphology in the Laminariales has been shown conclusively in L. digitata by Sundene (1962, 1964) and in S. polyschides by Norton (1969). John (1968) found in transplant experiments with L. hyperborea that wave action could affect morphology and Larkum (1972) has produced convincing evidence, from studying sheltered and exposed regions, that the lamina morphology changes with depth seen in L. hyperborea are brought about in response to changes in turbulence and wave action rather than light.

The data presented above suggests that light intensity is also important in controlling lamina morphology. However, Loose et al. (1934) and Myers (1946) have found that lower light intensities can cause increased multiplication of cells and extrapolation from plants of 1cm length to the field must be done with caution. (Kain, 1969). It is felt that light intensity may play some role in effecting lamina morphology changes but multifactorial experiments incorporating wave action are needed.

Itwas also found, that no changes in the ultra-structure of the chloroplasts of either deep growing or low light cultured plants occur. It is possible that the small increase in chlorophyll found in deep plants may well be

accomodated in an increase in the volume of the chloroplasts. The appearance of large amounts of polyphenols in the shallow growing plants may well be a reaction to the greater light intensities found there.

CHAPTER 9

TRANSLOCATION

Introduction

It has long been assumed that translocation occurs in Laminaria. Thus Fritsch (1945, p.234) states that - "both the trumpet hyphae of Laminariales as a whole and the sieve tubes of Macrocystis and Nereocystis are no doubt conducting elements", and, more recently Esau (1969, p.265) states that "both kinds of cells (i.e. trumpet hyphae and sieve tubes in Macrocystis) are assumed to be concerned with conduction".

Esau (loc cit) has reviewed the early anatomical investigations of these cells. The terminology of Smith (1939) is adopted here: thus the cells in the medulla of the stipes of Laminaria are called trumpet filaments (trumpet hyphae or trompetenzellen, Ziegler & Ruck, 1967) whilst the large 'true' sieve tubes of the Macrocystis type are called sieve filaments.

The trumpet filaments are long cells, interconnected by end walls which were interpreted as sieve plates by Oliver (1887) and Sykes (1908). The enlargement of the cells at these end walls is possibly caused by a longitudinal stretching of the cell during the elongation of the stipe (Smith, 1939; Nicholson, 1968). These cells are not part of a solid block of tissue but are embedded in mucilage. They were thus named "trumpet-shaped hyphae" (trompetenförmige Hyphen) and also "sieve hyphae" (Siebhyphen) with reference to the sieve-plate form of the end walls (Wille, 1885; Oliver, 1887).

Oliver (loc cit) found an early formation of callose and an eventual blocking of the sieve plates with this substance and pointed out that the sieve plates in the trumpet filaments have smaller pores than the sieve plates of sieve filaments. Ziegler & Ruck (1967) have examined the trumpet filaments with the electron-microscope. They examined the sieve plates of Laminaria species including L. hyperborea) and cameto the conclusion that the sieve plate was a single large pit-field (perhaps the largest in the plant kingdom) with a concentrated number of large pores. The trumpet filaments were found to be living

cells with normal organelles and a persistent nucleus. The cross wall consists of a single primary pit-field with 20,000 - 30,000 plasmodesmata of normal structure and dimension. They have a density of 50-60/ μm^2 and there were indications that the rim of the pores and the surface of the end wall is covered in callose. In a comparison of these structures with the sieve plates found in the sieve filaments of Pelagophycus and Macrocystis a progression was noted from the large numbers and dense packing of large sized pores (0.06μm) in Laminaria, through the intermediate Pelagophycus, with less numerous and dense but larger sized pores (0.3-0.8μm) to Macrocystis, with the longest cells terminating in a sieve plate with the least dense and numerous but largest pores (2-6μm).

Ziegler & Ruck (loc cit) concluded that the trumpet cells of Laminaria are conducting elements and as a primitive alga, long distance conduction has been mediated by a simple extension in the number of pores in the sieve plate. They speculated that the callose lining in the pores and sieve plate gave some mechanical support against a negative pressure in the cells caused by the mass flow of solutes. However, there is little evidence to suggest long distance mass transport occurs in Laminaria.

Experimental studies on translocation in the Laminariales include those of Bodenberg (1928), who found that the small movement of lithium salts (1-2 cm in 36 hours) in Nereocystis luetkana could be accounted for by diffusion, and Crafts (1939), who measured the rate of exudation from the cut end of stipes of Macrocystis and found that the exudate flowed at rates comparable to the mass transport in higher plant phloem. Sargent & Lantrip (1952) inferred that actively growing apical tips of Macrocystis could not supply their requirements for growth through their own photosynthesis and that the excess photosynthate from mature fronds was translocated to the sink regions in the tips, the holdfasts, and the young sporophylls.

Since then it has conclusively been shown that rapid, long-distance transport of organic compounds occurs in the stipes of members of the Lessoniaceae. Thus Parker (1963) showed that C^{14} labelled photosynthates moved through the stipes of Macrocystis at rates of at least 50 cm/h towards the apex and less rapidly towards

the holdfast. Parker (1965, 1966), using C14 tracer and fluorescein dye, found that both dye and C14 products move mainly in the sieve filament region acropetally and basipetally, with the fastest transport to the sink regions of rapidly growing api .es, and that the rate of transport of organic compounds can reach 65-78 cm/h. Fluorescein rarely moved faster than 1.0 cm/h. It was also noticed that fluorescein was found in the medullary (trumpet) filaments but the distance travelled was not as great as that in the perimedullary sieve filaments. A small amount of exudation was found from the medulla when the stipe of Macrocystis and other laminarialean algae (species not mentioned) was severed. Parker (1965) contended that the trumpet filaments may play at least a secondary role in translocation. Examination of the sieve tube exudates showed that over 90% of the radioactivity was in mannitol, which comprised 3.6% (w/v) of the total exudate. The chemical composition of the exudate and the similarity between the structure of the sieve tubes in Macrocystis and those in higher plants (Parker & Huber, 1965) - apart from the absence of companion cells in Macrocystis, whose sieve tubes contain numerous organelles and show a greater metabolic independence than the angiosperm sieve tube - led Parker (1966) to suggest that the translocation mechanism in Macrocystis was probably similar to that in vascular plants.

Nicholson (1968), using photosynthetically fixed C14 bicarbonate as tracer, found that mannitol is moved through sieve filaments at rates of up to 40 cm/h to the main growing points in Nereocystis luetkana. She felt that the trumpet filaments are not likely to move much material since a heavy callose deposit is present blocking the end walls and the lumen of these cells offers a high resistance to the flow of a translocation stream.

There have been fewer studies on translocation in the Laminariaceae. (Parker (1956) carried out experiments on the translocation of phosphate (P32) and fluorescein dye in Laminaria agardhii and Fucus vesiculosis. He found that the isotope never moved more than 1cm in 12 hours from the point where it was absorbed and often not even as much as that. Exudation from severed fronds and stipes was found to come from mucilage ducts and not from medullary filaments (see also Blinks, 1951). Trumpet filaments were often found to be in a state of cytoplasmic degeneration. In all, Parker concluded that no mass flow of these tracers occurred

in Laminaria, that the movement of dye and isotope occurred at rates no faster than the calculated rates of simple diffusion and that the elongated trumpet filaments bore no structural or functional resemblance to 'true' sieve tubes.

However, this work does not take into account any seasonal differences that may occur. Parker (1966) mentions that the translocation of inorganic ions appears to be difficult to demonstrate both in the Laminariaceae and in the Lessoniaceae (c.f., Bodenberg, 1928).

In Chapter 4 an 'in situ' growth experiment demonstrated the importance of the old lamina to the growth of the new lamina in the spring and Lüning (1969a, 1970a) has shown the stipe and old lamina contribute to the growth of the new lamina under field conditions and that the stipe may consume part of the reserves in the old lamina in darkness. It was concluded that the bulk of reserve materials supporting the growth of the new lamina were stored in the old lamina and it was calculated that about 30% of the new lamina area produced in the spring was due to these reserve substances.

The 'in situ' experiments described in chapter 5 indicated that the new lamina tissue was below compensation for photosynthesis in February but that after March - at least above the canopy - the growing lamina could photosynthetically assimilate adequate amounts of carbon for its growth. It was also apparent that the stipe tissue was often below compensation during the majority of the growing season even when the tissue was above the forest canopy. It is thus apparent that an alternative carbon supply to photosynthesis is required to support the growth of much of the tissues of Laminaria. The most attractive alternative is translocation from actively photosynthesising regions and Lüning (1971b), has demonstrated translocation of C^{14} labelled assimilates in vegetatively growing lamina tissue of Laminaria saccharina and L. hyperborea. This work showed that carbon 14 radioactivity moved from the old lamina to the new lamina of L. hyperborea, without much lateral movement. The movement was largely seasonal, with much more export of label in April than in October or January when little or no growth of the new lamina takes place. The main labelled substance was found to be mannitol (55-70% of total radioactivity) and the radioactivity was confined to the medullary region.

The rate of movement of this C14 'pulse' was found to be not greater than 8 cm/h in L. saccharina. In this species it was noted that an 'accumulation zone' existed at the base of the new lamina about 3-5cm from the upper part of the stipe where, after 48 hours, about 60% of the total radioactivity exported had accumulated and a further 10% of the total activity exported was found in the holdfast and stipe. The holdfast acquired slightly more label than the stipe. A 'transport zone' was defined as a zone from the old lamina to the accumulation zone where no accumulation of radioactivity occurs and a decreasing profile of activity occurs.

Lüning found that the basal zone of the lamina and, to a smaller extent, the holdfast, act as "sinks" for photosynthate assimilated in distal regions (the old lamina), providing that there is active vegetative growth in the lamina. The rate of movement shown was some 5 times lower than that reported for Macrocystis (Parker loc cit) and Nereocystis (Nicholson, loc cit). The major translocate, mannitol, is the same as that found in both Macrocystis and Nereocystis.

In the present study several experiments have been carried out on aspects of the translocation of labelled assimilates in L. hyperborea. These were carried out particularly to see whether the amounts of material translocated are sufficient to maintain the observed growth rate from field studies.

Results

Some of the data presented here consists of low counts above background cosmic radiation. In chapter 2 it was shown that, with the counting system used, counts of 1-2 cpm above background were significant. Nicholson (1968) arbitrarily selected a repeatable count of 5 cpm above background for the identification of the translocation front in Nereocystis luetkana.

Experiment 9:i Translocation from lamina to stipe and holdfast

Date 11.2.70

Details A plant, stipe length of 40 cm, including holdfast, aged from 4 to 5 years, was put into the apparatus shown in Figure 2:4 and the lamina was incubated with 100 μCi of sodium bicarbonate-C14 added in 1 ml to the seawater medium (21.) in the incubation chamber. The lamina was incubated for 47.5 hours at 9°C with an overhead

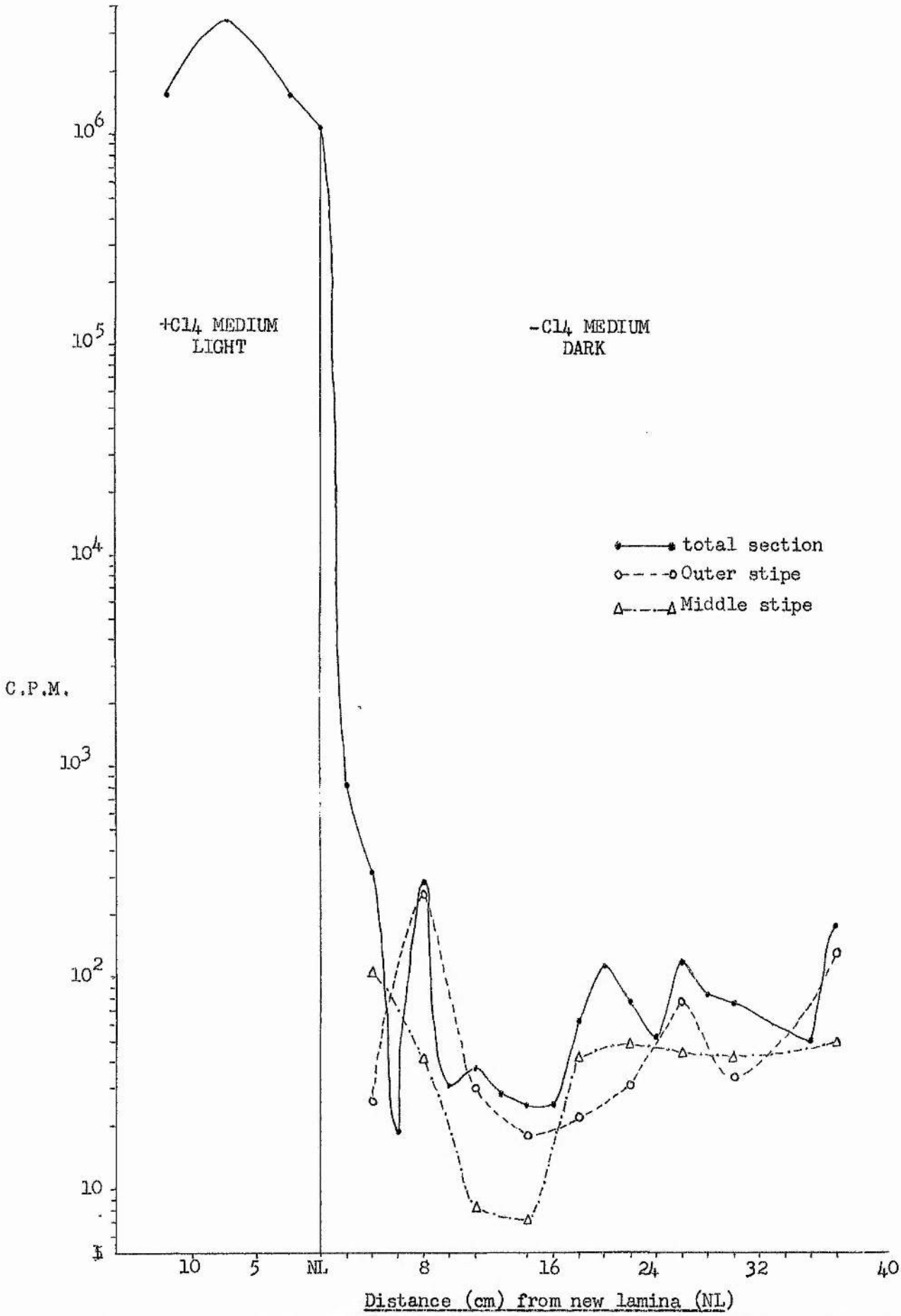


Figure 9:1 Distribution of radioactivity along the stipe after 47.5 hour incubation of lamina in sodium bicarbonate-C^{14}

irradiance of 3.13 cal/cm^2/h. After this exposure the lamina was severed at the transition zone and the lamina tissue cut into 5cm wide strips and killed in hot 80% ethanol. The stipe was cut into 2cm lengths with every alternate section being cut into and analysed separately as outer stipe (meristoderm plus most of cortex) and the middle stipe (medulla plus a few perimedullary cortical cells).

Tissues were fully extracted with ethanol and counted on planchets with the gas-flow system. Low amounts of radioactivity were found in the small aliquots (0.1ml) counted from these initial extracts so 5ml aliquots of the extracts were dried down in crucibles and taken up in small amounts of alcohol which were plated and counted. Results are shown in Figure 9:1.

As can be seen the profile of radioactivity fell off rapidly with distance down the stipe and counts are quite constant over the whole of the stipe. There was a slight indication of an accumulation of label at 7-8cm from the transition region, but this may not be significant. There was also an indication that the radioactivity accumulates in the holdfast. There was no indication of the route of transport of the radioactivity in the stipe. An estimate can be made of the quantity of dry matter thetranslocation of this amount of radioactivity represents.

Comparison between the mass transfer of mannitol and growth rate of stipe

It is assumed that the radioactivity mainly travels as C14 mannitol. Some indication that mannitol is the translocate is given in experiment 9:iii, Lüning e.a. (1971) has also shown that mannitol represents 55-70% of the translocate in his experiment with Laminaria.

The number of counts found in the stipe and holdfast can be converted into the total amount of mannitol moved into these regions during the course of the experiment using the specific activity of the labelled pool of mannitol in the lamina. This is calculated in the following way, using the data for the 0-5cm strip of old lamina:-

1. The radioactivity in the ethanol soluble fraction is contained in mannitol (Bidwell, 1958a).

2. The total mannitol content of this piece of lamina is 141.8 mg using the value for February in Figure 7:7 of 0.119 mg mannitol/mg dry weight and a total dry weight of 1189mg.

3. Assuming that total labelling of the mannitol pool in the lamina occurs in the 48 hour exposure then the specific activity of this mannitol is 1,546,900/141.8 or 10,909 cpm/mg dry weight of mannitol.

4. The total counts found in the stipe and holdfast were converted to mg mannitol using this specific activity, assuming that this value represents the specific activity of the mobile pool of mannitol.

Thus,

Total counts from stipe and holdfast = 2,271cpm

Quantity of mannitol moved = 2,271/10,909
= 0.21mg.

This represents a mass transfer rate of 0.1mg mannitol/day into the stipe and holdfast.

Growth rate of stipes

The growth rate of the stipes can be estimated from the biometric data. From Table 3:11 it was shown that up to 80.3g dry weight can be accreted in the annual growth of a 7 year old plant at 3.1m, with smaller annual increments in stipe biomass for other age groups. If it is assumed that the stipes and holdfasts have a similar seasonal pattern of growth as the lamina and a peak biomass is reached in October then a daily rate of growth can be found. The data for a range of age groups is given in Table 9:1.

Table 9:1

Mean daily growth rate of stipe and holdfast at 3.1m*
(mg dry weight/day)

Age (years)	Holdfast daily growth rate	Stipe daily growth rate
1	0.7	0.3
2	-	0.3
3	1.9	1.9
4	2.9	6.6
5	18.1	49.0
6	12.5	49.0
7	84.5	264.0
8	4.3	28.6
9	3.9	9.9
10	12.2	-

* Assuming an annual growing period of 304 days.

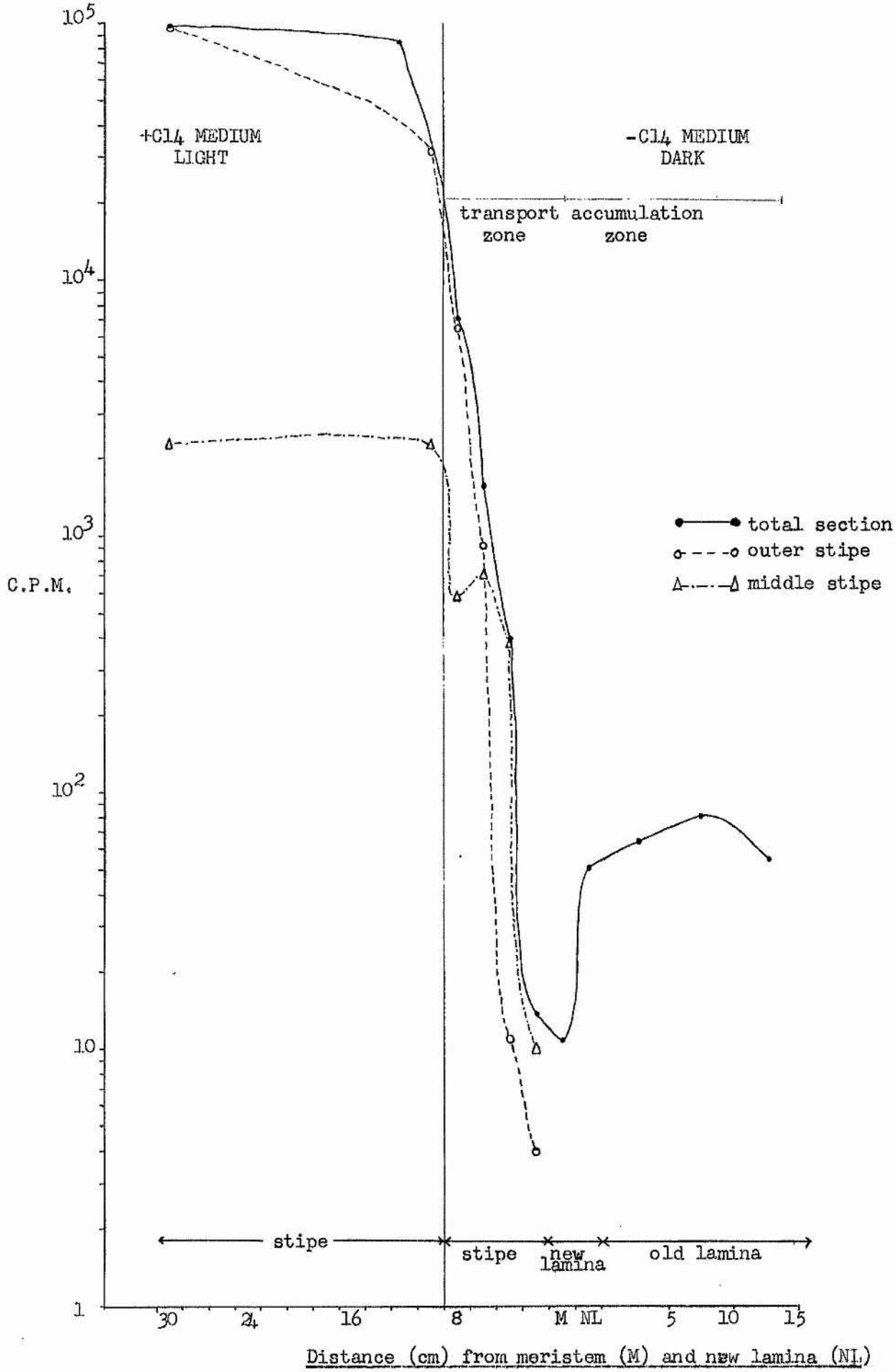


Figure 9:2 Distribution of C14 labelled translocate from stipe to lamina

The calculated mass transfer rate of 0.1mg mannitol/day could not support the estimated growth rate of the stipe of from 6 to 49mg dry weight/day from the above biometric data (4-5 years). However, the rates in Table 9:1 do not take into account any slower growth rate which is likely to occur in the stipes by analogy with the lamina 'lag phase' growth. It is possible that carbon requirements are much less than indicated during the first few months of the year.

Experiment 9:ii Translocation from stipe to lamina

Date 25.2.70. Temperature 8.5°C Incubation time 24 hours.

Details

The old lamina, new lamina (some 110 mg dry weight), and about 6 cm of stipe were wrapped in a black polythene bag and put into the lamina chamber of the large translocation apparatus. The whole of this was also wrapped to ensure darkness and no photosynthesis and thus to encourage flow to these 'sinks'. After incubation the plant was sectioned from the distal region of the old lamina to the holdfast as before and the results are shown in Figure 9:2.

The translocation profile falls off less steeply than that found in experiment 9:i in the opposite direction. Similar zones as those described by Lüning(1971) are apparent; thus a 'transport zone was found from the transition region between the light and dark incubation chambers to the meristem. This zone was about 8 cm long. After the meristem the radioactivity increased in an "accumulation zone". No estimate of the rate of transport could be made from this experiment but it gives data on the pathway of transport and the quantity of material exported to the lamina.

Pathway of transport

In the light incubation chamber (+ C^{14}) the outer cortex of the stipe and the outer holdfast tissue contained more activity than the middle tissue of both regions. Considerable lateral translocation of labelled assimilates from the photosynthetic meristoderm to the inner cortex and medulla had occurred within the 24 hour time course of the experiment.

In the 'sink' chamber, however, the situation was reversed and the activity in the medullary region exceeded that in the cortex. Even though there was a considerable amount of activity in the cortex it seems that the medullary region was a preferential pathway of transport of the radioactive assimilates.

Quantity of material translocated

The mass transfer rate of this translocation can be calculated as in experiment 9:1.

1. Total mannitol content of stipe section 10cm = 46.4 mg using the average stipe figure of 0.686 mg mannitol/mg dry weight (Figure 7:8).
2. The total ethanol soluble radioactivity in this section was 34,717 cpm.
3. Specific activity of mannitol pool = 34,717/46.4 cpm/mg mannitol
   = 748.2 cpm/mg mannitol.

The total counts found in the stipe and lamina region to the 'sink' chamber was 9,620 cpm and this represents 12.9 mg mannitol. Thus the mass transfer from the stipe to the lamina is 12.9 mg/day. The gradient of movement from the stipe to the lamina is much steeper than the opposite one and the quantity of mannitol moved is over 100 times greater than that moved from the lamina to the stipe.

Growth rate of the new lamina during the 'lag phase'

It is apparent from Figure 3:9 that the average increment of a new lamina of canopy plants from January to March is about 9g dry weight.

Thus the rate of mass transfer from the stipe quoted above at 12.9 mg mannitol per day would supply some 0.8g mannitol over the 2 month period from January to the beginning of March.

Experiment 9:iii The effect of dark starvation of 'sink' region on translocation

Date 14.3.70. Temperature 7.5°C Incubation time 51 hours

A plant collected on 5.3.70 was kept in an aquarium tank with only the old and new lamina wrapped in a black polythene bag to encourage a movement of photosynthate from the illuminated stipe to the growing new lamina kept in darkness. The experiment was set up in the large translocation apparatus (Figure 2:4) with 100 μCi injected into the stipe chamber. The sections were analysed for radioactivity and the results are shown in Figure 9:3.

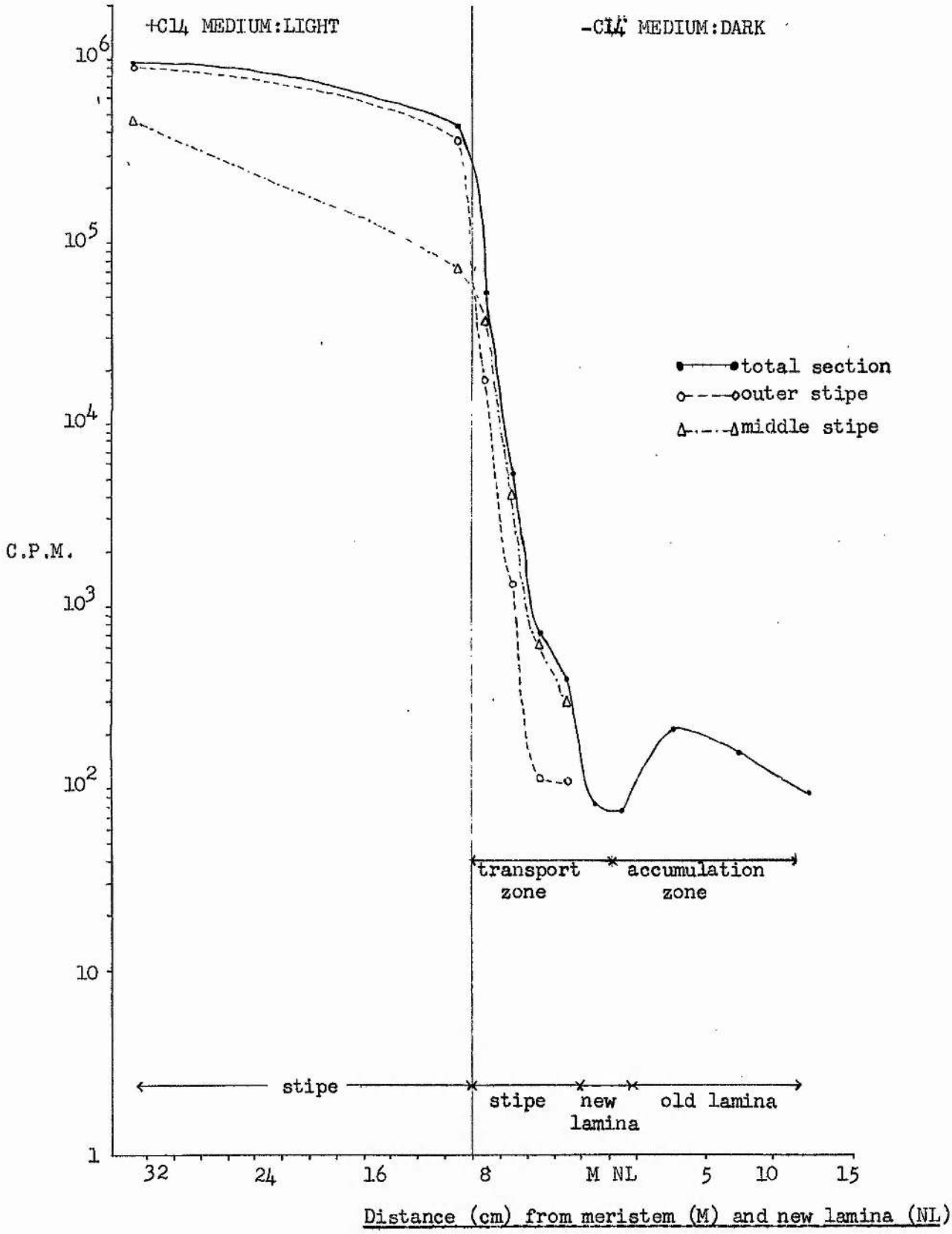


Figure 9:3 Distribution of C14 translocate from stipe to lamina

A slight increase in counts in the sink regions was seen compared with the data in experiment 9:ii and a higher profile was found along the sink region. An accumulation zone was again evident and occurred just distally to the new lamina region.

Nature of translocate

The radioactive substances moved laterally into the medulla in the source chamber and the radioactive materials translocated longitudinally along the stipe to the lamina were examined by paper chromatography. 5 ml aliquots from the medullary regions 2-4 cm; 4-6cm; 6-8cm; 8-10cm and 32cm from the meristem and new lamina were dried down on Whatman No 1 paper and run in the E.M.K. solvent. The major radioactive substance was found to be mannitol, from radiochromatographic analysis. Other unidentified peaks were found which may represent the amino and organic acids found in the translocate of Laminaria by Lüninge(1971) It is possible that mannitol is converted into other components 'en route'.

Quantity of mannitol moved

1. The total mannitol content of the stipe section 8-10cm from the meristem was 22.2 mg, using the average figure for stipe mannitol content in March of 0.49 mg/mg dry weight from Figure 7:8 .
2. The total ethanol soluble activity in this section was 442,395 cpm.
3. The specific activity of the mannitol pool is 442,395/22.2 cpm/mg mannitol; i.e. 19,955 cpm/mg mannitol.
4. The total counts in the stipe and lamina regions in the sink chamber was 62,693 cpm. This represents 3.14 mg mannitol.

This was moved in approximately two days so the rate of mass transfer from this experiment is about 1.6mg mannitol per day. This is somewhat lower than the previous value so it seems that dark starvation tends to decrease the amount of mannitol moved. At this rate of mass transfer the stipe would provide some 94 mg mannitol to the lamina in the period from January to 1st March.

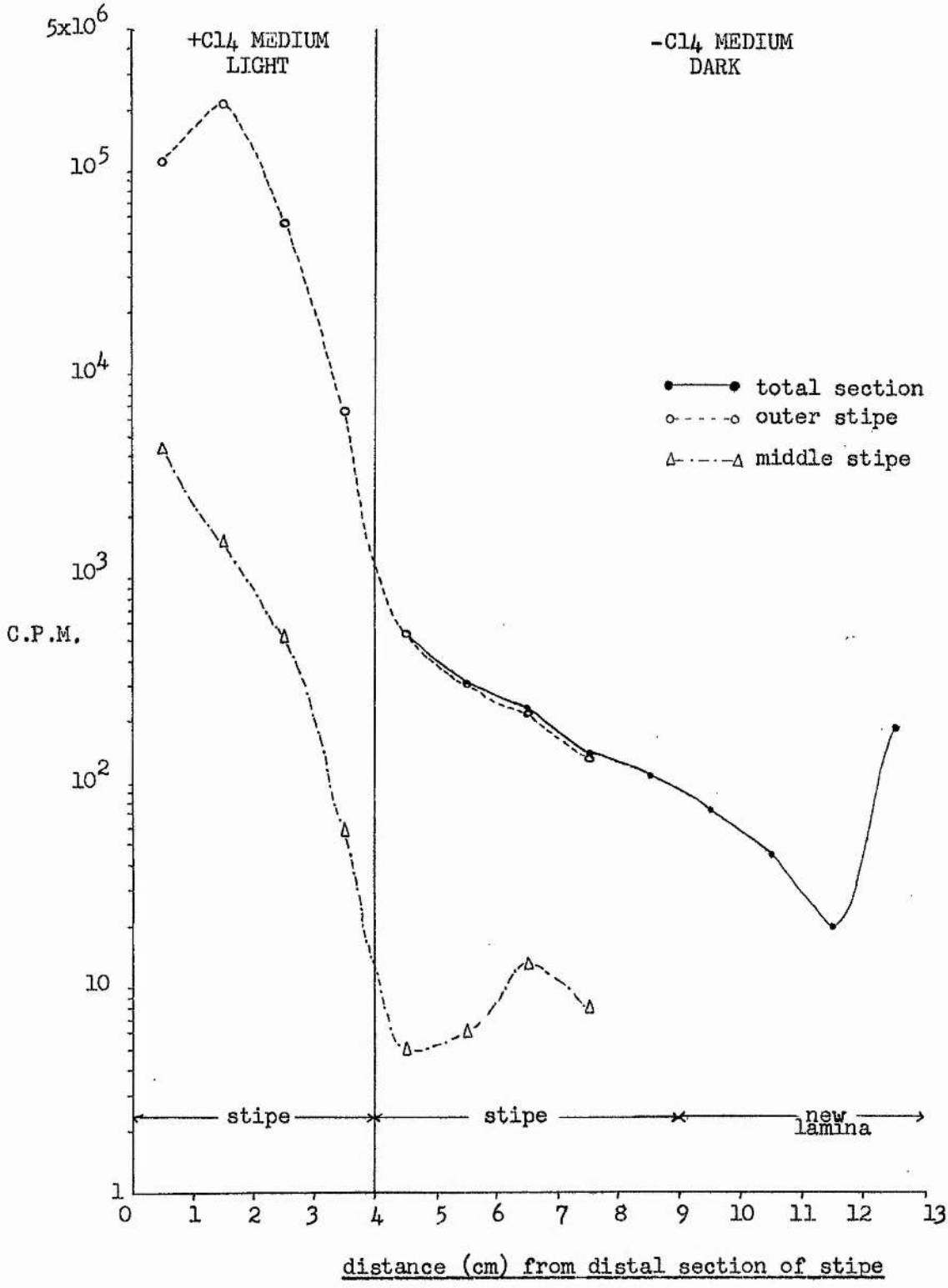


Figure 9:4 Distribution of C14 labelled translocate from stipe to new lamina

Experiment 9:iv Short term translocation from stipe to lamina

Date 20.4.71 Temperature 7.5°C Incubation Time 1 hour.

A small plant was starved for 5 days and the stipe was incubated in bicarbonate enriched seawater (+ 49 mg $NaHCO_3$/250ml) in the small apparatus (Figure 2:3) with 470 μCi C14 bicarbonate. The exposed tissue in the bridge region was frequently wetted. After the incubation the plant was cut up in 1 cm strips starting from a terminal stipe section which had been immersed in the incubation medium. The tissues were homogenised and counted in the liquid scintillation counter (L.S.C.). The results are shown in Figure 9:4.

Significant counts were obtained in the distal part of the new lamina after only one hour incubation. There was an indication of an accumulation of activity in the most distal section of the new lamina.

Rate of transport

Since significant counts were obtained at the most distal point during the one hour incubation it can be said that the tracer front moved at a velocity of at least 9cm/hour.

Quantity of mannitol moved

1. The total mannitol content of the first stipe section (0-1 cm) with a dry wt of 66.7mg was 21.9mg assuming an average mannitol content of the stipes in April of 0.329mg mannitol/mg dry weight (Figure 7:8).
2. The total counts in this section (although the whole tissue had to be analysed in the LSC, in this short incubation most of the label would be in mannitol) was 112,992 cpm.
3. The specific activity of the mannitol pool was therefore 112,992/21.9; or 5,159 cpm/mg mannitol.
4. There was a total of 1694 cpm in the sink region, which thus represented 0.33 mg mannitol.

This was moved into the upper stipe and new lamina in 1 hour so the rate of mass transfer was 7.9mg mannitol/day.

Overall it can be said that the stipe is likely to contribute between 2 and 13mg mannitol per day to the developing regions of the upper stipe and new lamina. This amounts to some 0.1 to 0.8g mannitol over the two months of January and

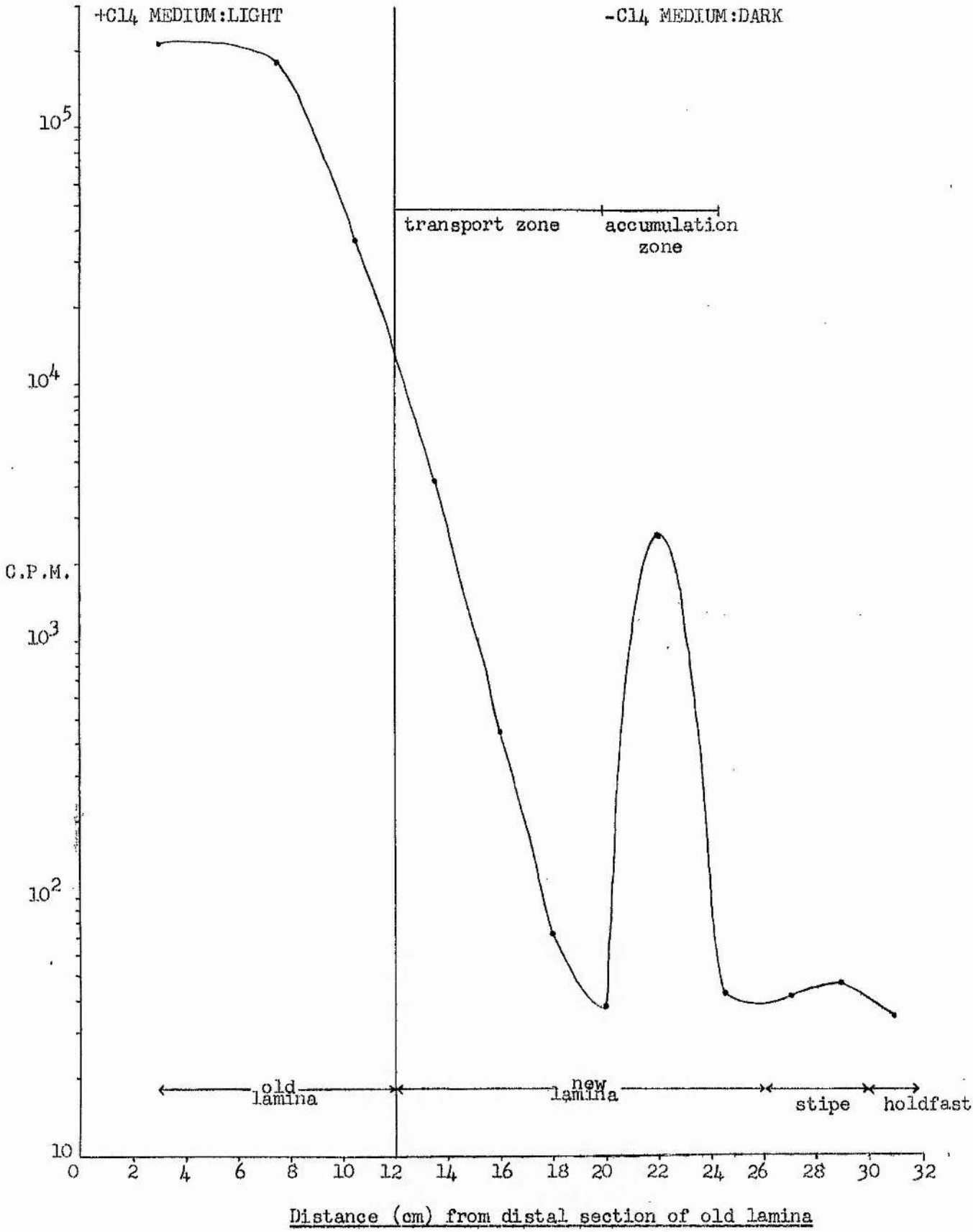


Figure 9:5 Distribution of C14 labelled translocate in new lamina, stipe and holdfast

February, when such supplies could be vital to new lamina development (see Chapter 5).

Experiment 9:v Translocation from the old lamina to the new lamina, stipe and holdfast.

Date 10.6.70 Temperature 7°C Incubation time 3 hours

Again using the small apparatus in Figure 2:3, the old lamina was allowed to assimilate Cl^4 sodium bicarbonate (30 μCi/150ml) in a bicarbonate enriched seawater medium (+29.4 mg $NaHCO_3$/150ml). The plant was then left in a dark wrapped box with fresh seawater flushing from the holdfast to the lamina for 20.5 hours. The tissue was then cut into small lengths and fully extracted with hot 80% ethanol. The results are shown in Figure 9:5 and the data for the sink regions of the new lamina, stipe, and holdfast are given in Table 9:2.

Table 9:2

Cl^4 radioactivity in new lamina, stipe and holdfast (cpm)

	Tissue	Length(cm)	EtOH soluble cpm in aliquot	EtOH soluble Total	Acid hydrolysate cpm in aliquot	Acid hydrolysate Total	Residue cpm in aliquot	Residue Total	Overall total
New Lamina	1	3	14	4095	7	112	15	52	4259
	2	2	1	420	1	22	3	8	450
	3	2		55	1	18			73
	4	2		18	1	20			38
	5	2	1	283	140	2338	11	40	2261
	6+M	3		3	43	43			43
Stipe	1				3	42			42
	2				2	35			35
	Holdfast				2	35			35

A steep fall in the radioactive profile was seen over the transport zone (terminology of Lüning, 1971b) and an accumulation zone was detected at the base of the new lamina near the transition region with the stipe. The stipe and holdfast did not accumulate much radioactivity.

Mass transfer of mannitol

The total amount of radioactivity accumulated in the new lamina, stipe,

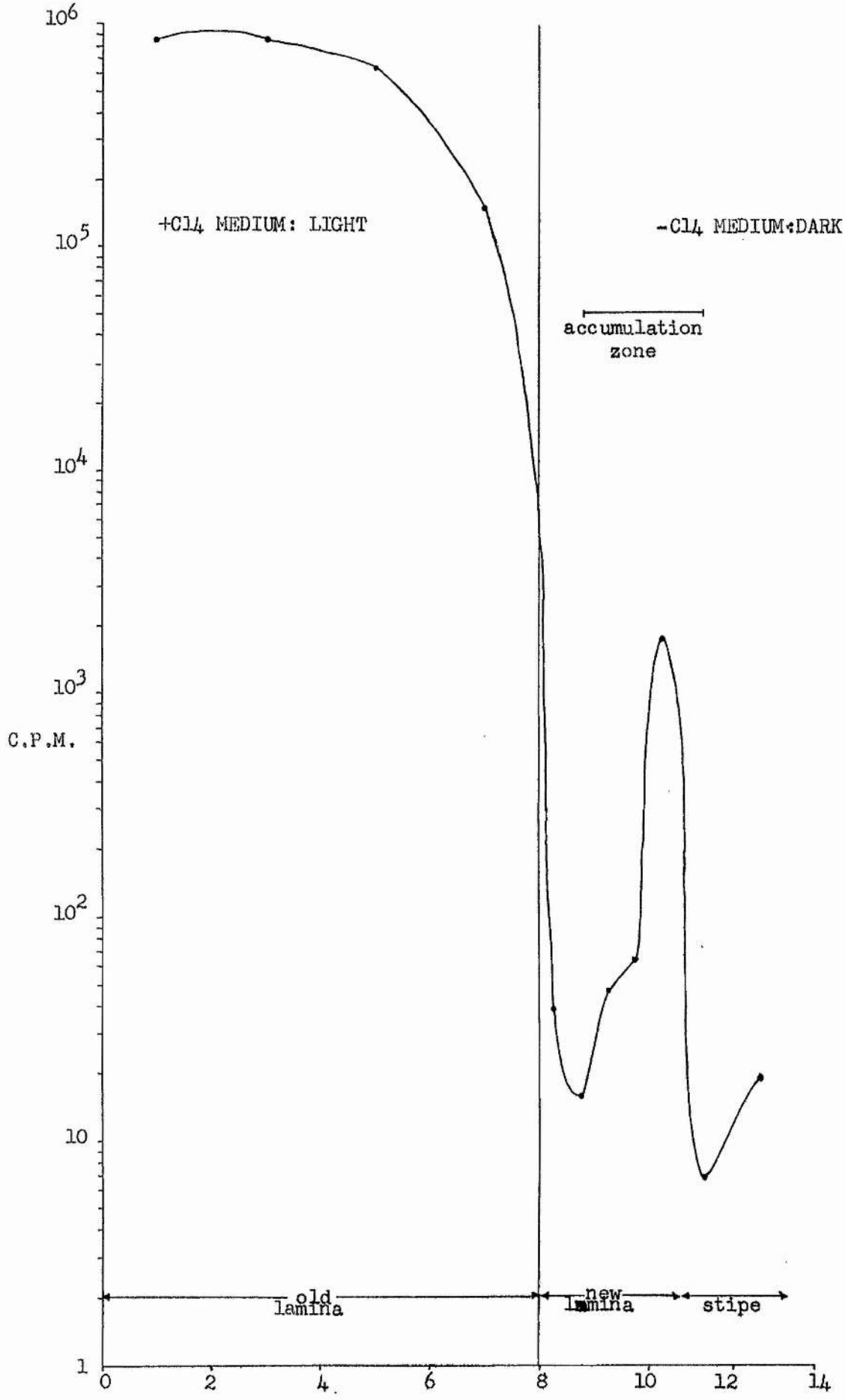


Figure 9:6 Distribution of radioactive translocate in thallus of L. hyperborea

and holdfast can be converted into the equivalent mass of mannitol moved using specific activity data from the old lamina.

1. The total mannitol content of the 3 old lamina sections analysed was 80.5mg, using the average content of the July 3.3m lamina from experiment C, Chapter 5, of 0.31 mg mannitol/mg dry weight.
2. The total ethanol soluble counts in these sections was 442,204 cpm.
3. The specific activity of the mannitol pool was 442,204/80.5 or 5,493 cpm/mg mannitol.
4. The total number of counts in the new lamina, stipe, and holdfast was 7648 cpm, which represents 7648/5493 mg or 1.39 mg. mannitol.

From this experiment it can be said that about 1.4 mg mannitol were moved into the sink regions in 20.5 hours. This amounts to about 1.6 mg mannitol/day and to 98 mg mannitol over two months. Thus, from January to the beginning of March the old lamina may be expected to translocate about 0.1g mannitol to the rest of the plant.

Experiment 9:vi. Translocation from old lamina to new lamina and stipe

Date 5.2.71 Temperature 9.5°C Incubation time 3 hours.

The data from this short time course experiment are shown in Table 9:3 and Figure 9:6. An autoradiograph of a similar experiment is shown in Plate VI; the new lamina was not adpressed to the film due to the bulkiness of the stipe but a degree of fogging was observed in the upper stipe which was significantly greater than that produced by a control strip of non-radioactive tissue. Similar autoradiograms have been presented as evidence of translocation of labelled assimilates by N. Ferrier (pers. comm.) and by Lüning (1971b). Chemography of the X-ray plates by these tissues, which exude mucilage when damaged, can be extensive and this method of interpretation of translocation is therefore suspect unless high activities and chemographic protection such as thin plastic sheet is used.

Table 9:3

Radioactivity in various fractions of the new lamina and stipe

Tissue	Section	Length (cm)	EtOH cpm in aliquot	EtOH Total	AH cpm in aliquot	AH Total	R cpm in aliquot	R total	Total
	1	0.5	5	10	2	33	1	5	48
	2	"	5	10	-	-	1	7	17
New	3	"	11	28	1	20	-	-	48
lamina	4	"	6	12	3	53	-	-	65
	5	"	902	1804	-	-	2	16	1820
	1	1.0	4	8	-	-	-	-	8
Stipe	2	"	6	12	-	-	1	8	20

Rate of movement

The velocity of transport of the isotope was calculated to be 4.5cm/3h or 1.5 cm/h if it assumed that the isotope front had only just reached the terminal section.

Mass transfer of mannitol

1. The total mannitol content of the old lamina section between 6 and 8cm was 45.9 mg mannitol assuming the old lamina mannitol content from Figure 7:7 in February to be 0.119 mg mannitol/mg dry weight.
2. The total ethanol soluble radioactivity in this section was 153, 750 cpm.
3. The specific activity of the mannitol pool 153,750/45.9 or 3343 cpm/mg mannitol.
4. The total activity in the sink region was 2026 cpm. This represented 2026/3343 or 0.61 mg mannitol. This had accumulated in the sink regions in 3 hours so the mass transfer of mannitol was 4.9 mg mannitol, per day. From January to the beginning of March the old lamina might thus be expected to translocate some 291 mg or 0.3g mannitol to the developing regions of the new lamina and stipe.

From this data and that in experiment 9 v it can be seen that between 0.1g and 0.3g mannitol could be provided by the old lamina, over the critical two month 'lag phase' period of new lamina development.

Experiment 9:vii Control experiment on the necessity for living tissue for the translocation from old to new lamina

In order to see whether the translocation observed from the old lamina to the new lamina requires living cells for the conduction of isotope a control experiment was carried out. It is possible that the movement observed was in 'free' space tissue since overall quantities and rates were low. Thus the maximum rate observed was 9 cm/h; this is about 4 times lower than the velocity recorded by Nicholson in Nereocystis and nearly 6 times lower than the rate for Macrocystis translocation (Parker, 1965). Translocation velocities, however, are notoriously difficult to interpret (Canny, 1960) but Peel & Weatherly (1962) found a rate of translocation of 30 cm/h in the willow using the aphid stylet technique so that the rates recorded for large brown algae are comparable to but smaller than values for higher plants. (See discussion; this chapter).

Details

The new lamina and stipe of a specimen of L. hyperborea were killed by immersing these tissues in boiling seawater taking care not to damage the old lamina. The old lamina was allowed to assimilate C^{14} bicarbonate in the usual way with 30 μCi Na HCO_3-C^{14} at 9°C for 2.5 hours and then transferred to running seawater for 17.2 hours.

Results

There was no detectable activity in any of the new lamina tissue. It would thus seem that living tissue is essential for the translocation observed in the experiments previously described.

DISCUSSION

Translocation of C14 labelled assimilates has been demonstrated in tissues of L. hyperborea. The translocation profiles from lamina to stipe, stipe to lamina, and old lamina to new lamina were characteristic in that they fell rapidly with distance along the gradient. This is unlike the situation in Macrocystis where high levels of radioactive translocate were found for considerable distances from the point source. There was some indication of an accumulation of translocate in basal regions of the new lamina near the transition region, in agreement with the data of Lüning (1971b).

The calculated velocity of movement was between 1.5 to 9 cm/h which is similar to the findings of Lüning who quotes rates of between 3 and 8 cm/h. The velocity of movement of the radioactive front can be compared with the theoretical rate of diffusion of mannitol in water by calculating the diffusion coefficient from the experimental data. Thus, the data from experiment 9:iv is used and assuming the 'source' region (stipe) is steadily supplying photosynthate to the 'sink' region (lamina), which is steadily utilising the supply, then Fick's First Law of diffusion applies.

Thus, over a linear concentration gradient,

$$\frac{dm}{dt} = -D_{obs} A \cdot \frac{C_1 - C_2}{x}$$

where

- dm = mass of mannitol moved
- dt = time course
- D_{obs} = experimentally derived diffusion coefficient
- A = cross sectional area of stipe
- C_1 = concentration of mannitol in the stipe
- C_2 = concentration of mannitol in the lamina
- x = distance moved

Rearranging the formula:

$$D_{obs} = \frac{dm \cdot x}{dt \cdot A \cdot (C_1 - C_2)} \quad cm^2/s$$

From experiment 9:iv.,

dm = 0.33 mg mannitol

dt = 3600 seconds

D_{obs} = unknown diffusion coefficient

A = 6.2 cm^2

C_1 = concentration of mannitol in the stipe section 0-1 cm. The value obtained of 21.9 mg (see experiment 9 iv) was based on dry weight but for the above equation the concentration is based on volume (w/v). Thus, assuming a fresh weight:dry weight ratio of 5.9, a water content of 83% fresh weight, and a water density of 1, the concentration of mannitol is 46.5 mg/cc. water.

C_2 = The concentration of mannitol in the lamina section 9-10cm was 12.8 mg, assuming a mannitol content of 0.12 mg/mg dry weight and a dry weight of 106.6 mg. This is converted to concentration on a volume basis using a fresh: dry weight ratio of 6.7, a water content of 85%, and a water density of 1. This gives a mannitol concentration of 15.2 mg/cc. water.

x = 8 cm.

Substituting the data,

$$D_{obs} = \frac{0.33 \cdot 8}{3600 \cdot 6.2 \cdot 31.3} \text{ cm/s}$$

$$= 3.7 \times 10^{-6} \text{ cm}^2/\text{s}$$

The value for D_{obs} can be compared with the quoted value of the diffusion coefficient of mannitol in water, i.e. 0.682 cm^2/s (Handbook of Chem. & Phys., 1967). It can be seen that a diffusional model cannot be ruled out for the translocation system investigated since the diffusion coefficient obtained from experimental data was some 2×10^{-5} smaller than the theoretical diffusivity of mannitol in water.

However, data in this chapter and the data of Lüning (1971b) suggested that the pathway of transport may be the medulla. If specific cells such as trumpet filaments were the pathway of transport then the cross-sectional area to be used in the equation would be smaller than the stipe value and the diffusion coefficient would be correspondingly larger. No estimate could be made for this adjusted value as evidence of the pathway of transport is lacking; once this is known a better estimate of the kinetics of this translocation system can be made.

It was indicated from two experiments that the translocate is mainly concentrated in the medulla. This suggests that the trumpet filaments may play some role in this movement. Parker (1965) contended that medullary (trumpet) filaments may play at least a secondary role in translocation. Parker & Huber (1965), however, said that the trumpet filaments were not homologous to the sieve tubes (in Macrocystis) and that they probably do not function in translocation in contradiction to previous views (Fritsch, 1945; Oliver, 1887). Nicholson (1968) felt that trumpet filaments do not conduct much material in Nereocystis since they were largely absent in the lamina and they had heavy 'callose' deposits with only a small lumen. However, it is possible that in these rapidly growing species with growing tips well below the light compensation for photosynthesis, there is little conduction in the small trumpet filaments compared with the large sieve filaments which offer less resistance to mass flow of solutes. Smith (1939) pointed out that the structure of the trumpet filaments in Laminaria doesnot resemble the large sieve tubes in Macrocystis and mentioned that they do not have true sieve plates but rather a large pit 'membrane'. Parker (1956) found that exudation from cut surfaces of the lamina and stipe of Laminaria, when stained with acredine orange and viewed under a microscope, came from mucilage ducts and not from trumpet filaments; he found many of these latter cells in a state of cytoplasmic degeneration, and concluded that they did not resemble "true" sieve tubes.

Ziegler & Ruck (1967), however, implicated such cells as conducting elements from anatomical considerations alone. It would appear from the data presented in this chapter that the medullary region forms at least a preferential pathway for the conduction of labelled assimilates. Lüninga(1971) found that most of the activity in the 'transport' zone in Laminaria was concentrated in the medulla and was also within distinct "channels", but the resolution of his autoradiographs was not sufficient to implicate any specific cells. It would seem most likely that the trumpet filaments were involved since these are the main constituents of the medulla and are longitudinally orientated

(Fritsch, 1945) as were Lüning's 'channels'.

In any consideration of translocation the mass transfer of solutes is the most meaningful measure which determines the importance of the process to the growth of the plant. Thus an estimate has been made of the amount of material, as mannitol, which was moved to the sink regions and this has been compared with the observed growth rates of these regions from the biometric data in chapter 3.

Thus it was found that the lamina could transfer about 0.1 mg mannitol per day to the stipe and holdfast. The biometric data suggested that the stipe and holdfast accreted much more than this. However, these growth rates,are calculated on the assumption that the maximum stipe biomass occurred at the same time as that of the lamina, and the biomass increment during the first few months of the year may be much less than this rate. It is possible also that stipes and holdfasts can photosynthesise to some extent, during the rapid growth phase; thus one 'in situ'(experiment (N), Chapter 5) showed a net carbon gain for stipes, and Lüning (p.comm) reported that stipes may be able to make up for respiration losses by photosynthesis.

Nevertheless most of the 'in situ' experiments showed that stipes could not photosynthesise enough carbon to balance respiratory losses. This was particularly so under the canopy. No comparable data is available on the carbon balance of the holdfast because photosynthetic rates could not be expressed on an area basis. However, Johnston (pers. comm.) has examined the chloroplasts of developing haptera and found that the plastids are often colourless and present as undeveloped proplastids. It is thus likely that the holdfasts do not photosynthesise efficiently especially as they are next to the bedrock and will not receive much light. The seasonal data on mannitol content suggested an accumulation of mannitol occured in the stipe and holdfast from December to February which does suggest that mannitol was moved from the lamina to the lower regions of the plant. Black (1950a) found a similar increase in the mannitol content of stipes at this time. Experiments described here, and the data of Hellebust & Haug (1969), show that considerable lateral translocation

can occur presumably via the pits in the side walls of cortical cells. Bisalputra (1966) showed that the pores in the primary pit fields of Egregia and Fucus contain protoplasmic strands and movement could occur through these pores. The high concentration of mannitol found in the middle stipe (Figure 7:8) may mean that the middle stipe acts as a storage region for the outer stipe and meristoderm when cell division and secondary growth occurs. Presumably the primary growth of the stipe represents a fraction of the rates of stipe growth observed in Table 9:1 so it is possible that translocation from the old lamina and photosynthesis could account for the primary growth of the stipe, and lateral movement from the middle stipe may account for the secondary growth, although there is no data available to test this hypothesis. Overall it would seem that there is not a high mass transfer of organic compounds in the stipe of L. hyperborea of the same magnitude as that found in Nereocystis, where some 1.2 g dry matter/day was found to be moved down the stipe (Nicholson, 1968).

The new lamina of canopy plants (7 to 10 years) was shown to accrete up to 9g dry weight by March. Whittick (1969) has shown that younger plants accrete much less dry matter in the early growth. Thus 4 to 5 year plants may form a lamina of between 1 and 3g dry weight by March. This gives a growth rate of between 16 and 50 mg dry weight/day. The rate of mass transfer from the translocation experiments showed that the stipe can provide up to 13mg mannitol/day to the new lamina and the upper stipe. The old lamina was shown to provide from 2 to 6 mg mannitol/day to the new lamina.

Thus it would seem that the old lamina and older stipe regions could provide enough metabolites for the development of a new lamina in plants up to 4-5 years. This is especially important since these plants constitute the understorey shaded individuals in the kelp forest. The data for mass transfer in older plants is not available since the experiments on translocation from the old lamina to the new lamina were restricted to small, young plants which would fit into the apparatus. It is likely that older plants would have greater concentrations of mannitol available for translocation and a higher mass transfer would occur in them, although Luning's (1970a) data suggest that older plants do not produce a large new lamina in darkness. It is also possible that photosynthesis can take

place at times from January to March if there are not constant storms; some of Kain's data (unpublished) has shown that the light levels during these months may exceed the compensation point calculated in chapter 6 for the new lamina. The importance of translocation from the old lamina to the new lamina is indicated from the 'in situ' growth experiment described in chapter 4 and by the work of Lüning (1969a, 1970a). However, amputation could physically damage the new lamina or the removal of the old lamina may mean a loss of hormones as well as carbohydrates for the new lamina, so that such results should be evaluated with some caution.

It appears that the translocation of materials from the old lamina and stipe to the new lamina is critical for the establishment of a viable meristematic region and the removal of these source regions has a cumulative effect which subsequently improved light conditions cannot rectify. It must be pointed out that the criterion of "growth in area" used by Luning is slightly misleading in that the area increase may not correspond exactly to the true increment in dry biomass, and in fact Figure 3:4 shows that during these first two months the dry matter as a percentage of fresh weight actually goes down and turgor uptake of water has been suggested by Robertson (1970) to explain the rapid expansion in area. There were, however, also significant biomass increments made during this time. It is felt that the prediction of Lüning (1970a) that translocates from the old lamina account for 30% of the growth of the new lamina is possibly excessive in view of his use of area increment rather than dry weight. Nevertheless, data in the present study clearly imply that the translocation from the old lamina is critical until photosynthesis can take over in March or April. The rise in growth rate indicated by the 'rapid growth phase' is probably the result of photosynthesis taking over from the much smaller provision of materials from translocation.

Chapter 10

General Discussion

Table 10:1 gives a summary of the biometric and growth parameters found in L. hyperborea.

Table 10:1

Productivity and growth data of L. hyperborea at two depths

(data are maxima unless stated and source of data, e.g. experiment code, is given where applicable.)

Parameter			3.1m	9.1m
Net annual primary productivity	metric tons/ha/yr.	dry	21.3	9.5
		organic	16.5	8.0
Growing season mean productivity	g/m^2 sea-bed/day	dry	7.0	3.1
		organic	5.3	2.7
		carbon	2.6	1.3
Biomass	kg/m^2 sea-bed	fresh	36.7	11.3
		dry	5.8	1.7
		organic	4.7	1.5
Mean LAI	m^2 lamina/m^2 sea-bed		7.3	3.4
Gross photosynthesis	µg carbon/cm^2 lamina/hour		17.3 (N)	7.9 (B)
Net photosynthesis	g carbon/m^2 lamina/day		1.84 (N)	0.5 (B)
Assimilation number	mg CO_2/mg chlorophyll/h		1.75	0.74
	mg O_2/mg chlorophyll/h		1.27	0.54
Photosynthetic efficiency	total irradiance		2.4	5.8
	50% available irradiance		4.8 (D)	11.7 (Data 10.7m)
Saturation point	(cal/cm^2/h)		1.66	0.5
Compensation point (5°C)			0.45	0.35
Chlorophyll content	mg/g fresh weight		0.34	0.42
	mg/dm^2 lamina		3.6	3.9
Chlorophyll index (g chlorophyll/m^2 sea-bed)			2.6	1.3 (10.7m)

The data compiled in Table 10:1 indicate the high productivity of L. hyperborea and compare favourably with data for various aquatic, marine, and terrestrial communities given by Penfound (1956), Odum (1959), Ryther (1959), Newbould (1963), and Westlake (1963). Bray et al. (1959) estimate the productivity of a Typha reed-swamp at 16.8 metric tons dry matter/hectare/year and Penfound (1956) quotes a growing season productivity for Typha latifolia of 4.15 g carbon/m^2/day. Such emergent vegetation is considered to have a relatively high productivity (Newbould, 1963) and the data for Laminaria hyperborea suggest similar levels of annual productivity in this sublittoral plant.

The chlorophyll content, assimilation number (Willstatter & Stoll, 1918), and chlorophyll index are useful parameters in assessing the productivity of a community. Odum et al. (1958) have presented data for a variety of communities. The chlorophyll index of whole euphotic zones in well lighted seasons ranges from 0.1 to 3.0 g chlorophyll 'a'/m^2, and the assimilation number of such whole communities ranges from 0.4 to 4.0 g O_2/g chlorophyll'a'/h.

The data for L. hyperborea in Table 10:1 are based on total chlorophyll (a + c) so that the values on a chlorophyll 'a' basis would be higher. Thus the value of chlorophyll index at 2.6g chlorophyll/m^2 sea-bed (3.1m) and the value of the assimilation number at 3.1m i.e. 1.27mg O_2/mg chlorophyll/h are within the range of the 'stratified communities' defined by Odum et al. (loc cit) as being 'the most productive yet measured'. The data for L. hyperborea may be compared with the chlorophyll index of 0.74 g chlorophyll 'a'/m^2 seabed, the chlorophyll content of blades of 0.21 mg chlorophyll 'a'/g fresh weight, and the assimilation number of 1.0 gO_2/g chlorophyll 'a'/h in Macrocystis. (McFarland & Prescott, 1959). Macrocystis has a comparatively low productivity of from 10 to 15 metric tons organic matter/hectare/year (Aleem, 1956).

It would appear that L. hyperborea is a highly productive alga. The sublittoral marine environment may, at first glance, seem to be unfavourable for plant life because of the rapid attenuation of light with depth, but it is probable that conditions are generally less changeable than in the littoral zone

where the overriding importance of tidal rhythms imposes a daily flux in temperature, exposure to desiccation etc. (Lewis, 1964). Ryther (1959) has indicated that the high production of benthic algae is probably due to the almost maximal absorption of light in dense seaweed beds and an almost continuous replenishment of nutrients due to currents and wave action. Chromatic adaptation undoubtedly is one main factor in the successful growth of macrophytic sublittoral algae such as L. hyperborea. The absorption maximum of fucoxanthin at 520nm (Tanada, 1951), is ideally suited for absorption of light in the coastal waters around Northern Europe where the maximum transmission of light is from 500nm to 550nm (Coastal Type 4, Jerlov, 1951). That fucoxanthin can transfer energy to chlorophyll has been demonstrated by Dutton & Manning (1941), Wassink & Kersten (1946), Haxo & Blinks (1950), and Tanada (1951).

The low values of irradiance recorded beneath the Laminaria canopy, where up to 93% of the ambient light above the canopy was cut off, in agreement with Kitching (1941), indicate the almost maximal absorption of incident light by the lamina of L. hyperborea. Neushul (1957) found that one lamina thickness of Macrocystis absorbs about 33% of incident green light and McFarland & Prescott (1959) recorded zero percentage transmission of white light at one metre depth below a thick Macrocystis canopy. Blackman & Black (1959) came to the conclusion that, providing nutrients and water are not limiting, the rate of growth of any community characterised by a high leaf-area index is dependent on incoming radiation. The high LAI recorded for L. hyperborea indicates that almost total absorption of incoming radiation occurs 'in situ'.

The high absorption of light by the lamina tissue of L. hyperborea is correlated with the high chlorophyll concentration and Plates II to V indicate the large size of the chloroplasts relative to cell size. It was noticed in some sections of the lamina that chloroplasts were present in the inner cortical tissue as well and Hellebust & Haug (1969) have indicated the high photosynthetic capacity of excised outer cortex and inner cortex plus medulla tissue of the stipe of L. digitata. These authors suggested that little photosynthesis could occur in these deeper tissues 'in vivo' due to the

absorption by the meristoderm layer. It is possible, however, that the diffuse light underwater (Jerlov, 1963) may reach these subepidermal chloroplasts and some fixation may occur in these regions. Thus the high and photosynthetically effective absorption of light by L. hyperborea can be correlated with the presence of fucoxanthin, the high concentration of chlorophyll, the dense packing of chloroplasts in cells, and the relative lack of 'nonreactive' opaque cell materials. Strain, (1950) has indicated the importance of chloroplast volume relative to cell volume and the presence of opaque structural material; the highest photosynthetic efficiencies are found in cells rich in pigments with large chloroplasts filling most of the cells. It is apparent that L. hyperborea possesses high light trapping ability. The reason why the productivity at 3.1m is not greater than it is may be the low light - saturation point of the lamina; thus the old lamina saturation point was 1.66 cal/cm^2/h or 342 ft-c (Chapter 6) and this compares with the saturation point of 1100 ft-c for Macrocystis at 25°C (Clendenning & Sargent, 1957), 1,000 ft-c for most 'shade' plants and 2,000 to 3,000 ft-c for most crop plants (Blackman & Black, 1959). One cal/cm^2/h is approximately equivalent to 206 ft-c (see Chapter 2). The irradiance at 3.1m may reach from 1,277 ft-c (6.2 cal/cm^2/h; experiment N, chapter 5) to 2,637 ft-c (12.8 cal/cm^2/h; experiment C, chapter 5). It is possible that the low saturation value limits the potential production of L. hyperborea in shallow water. Another reason for limited productivity is the self-shading that occurs in the dense populations at 3.1m indicated by the high LAI found there and the reduced size of young plants at 3.1m as compared with plants of the same age at 9.1m.

Growth at various depths

It was noted in Chapter 3 that the biomass and density of plants are reduced with increasing depth. Kain (1971a) has indicated that sub-optimal irradiances for the growth of gametophytes and early sporophytes may exist at the lower limit (15m) of L. hyperborea during the winter. Grazing pressures are a further hazard at depth and may reduce the density of dark surviving gametophytes (Kain, 1964) before light conditions improve in the spring.

Densities of plants at depth reflect the climatic conditions and grazing pressures present at the time of establishment.

Once established, it is clear from the biometric and photosynthesis data in Chapters 3 and 5 that the growth of an individual plant is primarily controlled by light intensity. Thus the growth of a plant ultimately depends on photosynthesis and the changes in the rate of photosynthesis at various depths and under the forest canopy indicated that light intensity was the main factor involved in controlling the photosynthesis rate.

It was noted in Chapter 3 and by John (1967), Kain (1967, 1969b), and Lüning (1969b, 1970a), that the growth of an individual plant may not be significantly reduced over the normal depth range. The photosynthesis measurements in Chapter 5 indicated the 'euryphotic' nature of L. hyperborea. Norton (pers. comm.) has found plants of L. hyperborea growing in caves at an estimated 4% of surface light. Kain (1967) quotes a cave population of L. hyperborea in the Isle of Man where growth seemed to be reduced by water movement rather than by the reduced light intensity.

In Chapter 5 it was shown that photosynthetic efficiency increased with depth and it is likely that this is an important survival feature in deep-growing plants, and may explain how a deep-growing plant can often grow to a size similar to that of shallow plants. It is possible that this increased efficiency is based on adaptations in deep growing plants similar to those found in 'shade adapted' plants, i.e. reduced compensation point and respiration rate and increased chlorophyll content and SLA compared with 'sun'plants (Björkman & Holmgren, 1963; Hughes,1966). Most of these features were exhibited by deep-growing plants (see Chapters 3,6,7, and 8). However, it is not certain whether deep-growing plants have the ability to modify these morphological and physiological characterstics to their advantage.

Thus the reduced compensation point at depth is a consequence of the reduced respiratory loss. The respiratory rate, in turn, was determined on a weight basis so that both th these features are determined by the 'thinner' lamina at depth. It was shown in Chapter 8 that SLA may be controlled by

light intensity; however, Svendsen & Kain (1971) and Larkum (1972) have correlated changes in the lamina morphology of L. hyperborea with wave action. It is possible that the changes in morphology are an integrated response to both light and wave action; whatever the causal factors in determining lamina morphology it would seem that the end result is beneficial to growth at depth since a reduced compensation point and respiratory loss results from the thinner lamina.

In Chapter 8 it was noted that chlorophyll concentration on a weight basis increased with depth and presumably with light intensity. It is not clear whether this feature has adaptive significance. Gabrielsen (1948) showed that photosynthetic capacity is directly proportional to chlorophyll concentration only up to 4mg chlorophyll/dm^2. Although chlorophyll content on an area basis in L. hyperborea was within this range it remained virtually unchanged with depth (Table 10:1).

Further, it was noted in Chapter 6 that deep growing plants do not have increased photosynthetic efficiencies at low irradiance compared with shallow growing plants (although the conditions of illumination in those laboratory experiments were not the same as found 'in situ'). This is unlike the true 'shade' plants of Bjorkman & Holmgren (1963), which had an increased photochemical capacity compared with 'sun' plants at low irradiance and consequently have an inherent advantage for existence in shaded habitats. Chapman (1948) has summarised the earlier work of Montfort (1934) who suggested that 'sun', 'shade', and 'deep water' forms of L. hyperborea exist and that these forms are correlated with variations in the lower depth limit of the species at different latitudes.

The data in this thesis does not indicate that deep growing plants of L. hyperborea are shade-adapted 'ecotypes'. Kain (1969) found that there was no significant difference in the growth of gametophytes and early sporophytes of L. hyperborea from the Isle of Man and various Norwegian strains, including L. hyperborea f. cucullata (Svendsen & Kain, 1971) at low irradiance and a range of temperatures. This substantiates the above conclusion that the

morphologically modified deep water plants of L. hyperborea are not shade adapted plants.

Survival at depth may be a result of the high light trapping ability of the species and it is possible that the increased chlorophyll content may assist in the maintenance of a high photosynthetic efficiency. Terborgh & Thimann (1964) correlated changes in the efficiency of growth of Acetabularia crenulata at different photoperiods with the higher chlorophyll content of cells grown in short days. Some secondary advantages may acrue from the increased SLA at depth. Light is confirmed as the key factor limiting the plant over its depth range.

Seasonal growth

The seasonal growth of the lamina of L. hyperborea was shown to occur in 3 phases; a 'lag' phase in which a small increment in lamina biomass occurred in the first few months, a rapid growth phase in which most of the lamina biomass was accreted and a 'slow' growth phase during the summer when very little growth took place. It is now known what 'triggers' off the growth in January. It may not be light since an 'in situ' experiment showed the new lamina to be below compensation at that time. Gessner (1955) has suggested that nutrients may be important in initiating this growth since the concentration of nutrients is high at this time of year. It is also possible that low temperatures may be involved in starting this growth. The growth experiment in Chapter 4 and the translocation experiments in Chapter 9 indicated that the old lamina and stipe can probably support the new lamina during this early growth.

The findings from the translocation experiments are summarised as follows:-

(a) the profile of the translocation gradient fell off rapidly with distance

(b) the velocity of movement of the radioactive 'front' was small. A diffusion model could not be ruled out for this rate of mass transfer.

(c) the preferential pathway of movement seemed to be in the medullary region of the stipe.

(d) the major labelled substance in the translocate was mannitol

(e) the mass of mannitol moved from the old lamina (2-6 mg/day) and the stipe (13 mg/day) to the new lamina could account for the growth of the new lamina during the first 2 to 3 months of the year.

(f) the growth of the stipe and haptera may be accounted for by translocation although transfer of substantial amounts of C^{14} mannitol was not evident in the stipe. The translocation system in Laminaria would appear to be primitive compared with that in the Lessoniaceae. This is probably associated with the difference in growth rates between the groups. Laminaria does not produce a large new lamina in the lag phase of growth and hence does not require a lot of material translocated to the new lamina. Data are lacking about the actual pathway of transport but from the experiments in Chapter 9 and the work of Lüningea(1971) it is likely that the trumpet filaments in the medulla are the conducting cells. If this is so the small pores and lumen of these cells would explain the low rates of movement found. It is clear that translocation is important in establishing a viable meristem in the early months of the year. The rapid growth of the lamina starts in March at 3.1m and in May at 9.1m. This period of growth is characterised by a high photosynthesis to respiration ratio. In the summer the photosynthesis rate declines and growth slows up. Lüning (1970a) has shown that the growth of the lamina of L. hyperborea ceases in July at 2m depth, whereas a small increase in area was noted in specimens at 6m depth.

The lag in the commencement of rapid growth and the slowing up of growth in deep plants compared with shallow plants suggests that controlling factor(s) show a similar time differential over the depth range of the alga. Black & Dewar (1949) and Gessner (1955) have suggested that the seasonal growth of the lamina is largely controlled by nutrient availability. Nutrients may be at different concentrations over the depth range of the alga in the summer if thermoclines are set up. The temperature data from the 'in situ' experiments showed that temperatures never varied more than $1^{\circ}C$ over the depth range of the alga and Kain (1971b) has reported a lack of shallow thermoclines off the Isle

of Man. If there is vertical mixing of water during the spring and summer then it is unlikely that a difference in nutrient content of water exists at the upper and lower limits of L. hyperborea. Thus it is improbable that the lag in the commencement and cessation of lamina growth of deep plants compared to shallow plants is due to nutrient availability at the two depths.

Light intensity may be a more important factor since irradiance is likely to improve during the spring at depth after it does in shallow water due to the rapid attentuation of light with depth. This may account for the time lag found in growth in deeper water.

With increasing temperatures the respiration rate was shown to decline and it is possible that damage to biochemical systems occurs at high temperatures. If this is the case then the decline of photosynthesis in the summer may also be explained as a temperature effect on enzymic reactions of photosynthesis. Clendenning & Sargent (1957) reported much reduced photosynthesis rates for Macrocystis at high summer temperatures. The temperature intolerance for growth of various Laminaria species has been shown by Tikhovskaya (1940) in L. saccharina, by Tseng et al. (1957) in L. japonica, and by Sundene (1962, 1964) in L. digitata. The deep growing populations of L. rodriguezii found by Fredj (1969) at 85m to 90m off Corsica were found to be existing in a stable thermal zone whilst still receiving sufficient light for growth.

It would appear that L. hyperborea is a stenothermal plant, intolerant of high temperature but capable of photosynthesis at a wide range of light intensities. Seasonal growth appears to conform to the classical growth pattern outlined in the Harder: Kniep theory, whereby rapid growth in benthic algae in colder waters is explained by the high photosynthetic capacity at low temperatures and light intensities. Doty & Archer (1950) and Morris (1959) have emphasised the importance of key factors and, in particular, the extremes of these factors which control the growth of organisms. It is probable that light and water temperature are the key factors controlling the seasonal growth of L. hyperborea.

Studies on the carbohydrate metabolism of L. hyperborea indicated that

mannitol was the primary substrate of respiration and that mannitol and laminarin were, to some extent, interconvertible. This data is in agreement with that of Yamaguchi et al (1966) for Eisenia bicyclis but, unlike these authors, the amounts and specific activity of glucose residues of laminarin did not decrease markedly during dark incubation. Laminarin, represented by the specific activity of glucose in acid hydrolysates, appeared to be built up during dark incubation with a concomitiant decrease in the specific activity of mannitol.

Carbohydrate studies also indicated that seasonal gradients of mannitol occurred in tissues of L. hyperborea which may be correlated with a translocation of mannitol to growing regions. Hetrotrophy was investigated and in all tissues examined the amount of C^{14} glucose retained was small, little metabolism of this substrate occurred, and glucose was not converted into mannitol. The haptera showed the highest heterotrophic potential.

Projected studies

In view of the data presented in this thesis it would be of interest to examine the following aspects of the growth of L. hyperborea:-

a) the importance of light and wave action in the control of lamina morphology using multifactorial experiments and 'in situ' transplant studies.

b) the heterotrophic potential of haptera and stipe tissue using a range of organic substrates since 'in situ' C^{14} experiments indicated a low photosynthetic capacity in these tissues.

c) translocation of assimilates from the old lamina and stipe to the new lamina of older plants.

SUMMARY AND CONCLUSIONS

A SUMMARY

1. The net annual primary productivity of L. hyperborea has been estimated from biomass increment croppings and biomass: age relationships. The values of 16.5 mt organic matter/ha/year at 3.1m and 8.0mt organic matter/ha/year at 9.1m indicate the high productivity of this species.

2. Various other growth parameters such as maximum biomass, LAI, net assimilation rate, chlorophyll content and index, and photosynthetic rates have been compared with the data for other communities.

3. An 'in situ' technique, which seems to measure a value close to gross photosynthesis in L. hyperborea, has been used to estimate the photosynthetic capacity of tissues of L. hyperborea at various depths, under the forest canopy, and during the seasonal growth with the following conclusions:-

a) Photosynthetic efficiency was found to increase with depth

b) The forest canopy had a marked shading effect on the photosynthetic capacity of tissue sections.

c) Photosynthesis measured during the season showed that growing lamina tissue was below the compensation point in February, that maximum photosynthesis was in May, and that photosynthesis decreased in the summer.

d) The values for daily net photosynthesis compared favourably with estimates of net assimilation rate from biomass studies.

4. Laboratory investigations on photosynthesis gave the following saturation and compensation data:-

		Compensation point ($cal/cm^2/h$)		Saturation point ($cal/cm^2/h$)
		5°C	10°C	
Shallow plant	Old lamina	0.45	0.65	1.66
	New lamina	0.498	-	0.498
Deep Plant		0.35	0.47	(0.498)

The new lamina of shallow plants was found to have a higher photosynthetic efficiency than the old lamina of shallow plants at low irradiance.

The old lamina was found to have a slightly higher photosynthetic efficiency at low irradiance than the lamina of deep growing plants.

5. Respiration rates were measured at various temperatures and the stenothermal nature of the lamina tissue was shown in the RT curve. Lamina tissue was found to have a low optimum temperature for respiration of 9.5°C. The respiration rate of deep-growing plants appeared to be lower than shallow plants. Mannitol wasimplicated asthe primary respiratory substrate and was interconvertible with laminarin; the latter did not appear to be utilised as suggested by Yamaguchi et al. (1966). There appeared to be little heterotrophic uptake and utilisation of exogenously supplied glucose but differences in uptake rates were found between tissues, that of the stipe and holdfast being greater than the lamina.

6. Young sporophytes maintained in culture showed that low irradiance resulted in a thinner lamina than that found in high light grown plants and light was implicated as an additional factor to wave action in the control of lamina morphology 'in situ'. Deep-growing plants which also had a thinner lamina than shallow plants, had an increased chlorophyll content on a weight basis but not on lamina area basis compared with shallow plants. The increased chlorophyll content was not accomodated by changes in the internal ultrastructure of the chloroplasts.

7. Evidence was presented for a translocation of C^{14} labelled assimilates from the old lamina to the new lamina and stipe and from the stipe to the lamina. The rate of movement varied from 1.5 to 9cm/h. The mass transfer varied from 0.1mg mannitol/day from the lamina to the stipe and from 13mg mannitol/day from the stipe to the new lamina, and from 2 to 6mg mannitol/day from the old lamina to the new lamina. These amounts were considered to be capable of supporting observed growth rates of the new lamina from biometric data.

CONCLUSIONS

1. The high productivity of L. hyperborea has been explained in terms of the high light absorption capacity of the plant.

2. Deep-growing plants are probably not true 'shade-adapted' plants although

many of the features of shade plants were shown. It is possible that the slightly increased chlorophyll content at depth may help to maintain the high photosynthetic efficiency noted at depth. The 'euryphotic' nature of the plant was emphasised.

3. Translocation was considered to be of some benefit in producing a small new lamina before the major growth occurred with the onset of high rates of photosynthesis

4. Light and temperature were recognised as the two 'key' factors in controlling the growth of the alga.

Addendum

The respiration data used to correct photosynthesis measurements in 'in situ' and laboratory experiments was derived from a set of experiments in November, the results of which showed considerable variation (probably inherent in the plant material), a feature also noted by Lüning (1971) and Kain (pers. comm.).

The data of Lüning (1971) did not become available until after completion of this thesis and this showed that a seasonal adaptation occurs in the photosynthesis and respiration of lamina tissue of L. hyperborea, with the greatest assimilatory surplus in the summer and a decrease in respiration rate on a weight basis towards summer. Unpublished data of Kain and Hopkin (pers. comm.) have also shown a fall in respiration rate on a weight basis towards the summer.

It would appear, from this evidence, that the respiration rates used in this thesis are high, especially for 'in situ' experiments in the summer. This may explain why the net photosynthesis values found are low and also why the compensation data reported in Chapter 6 are higher than the data of Lüning (1971).

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PLATE 1

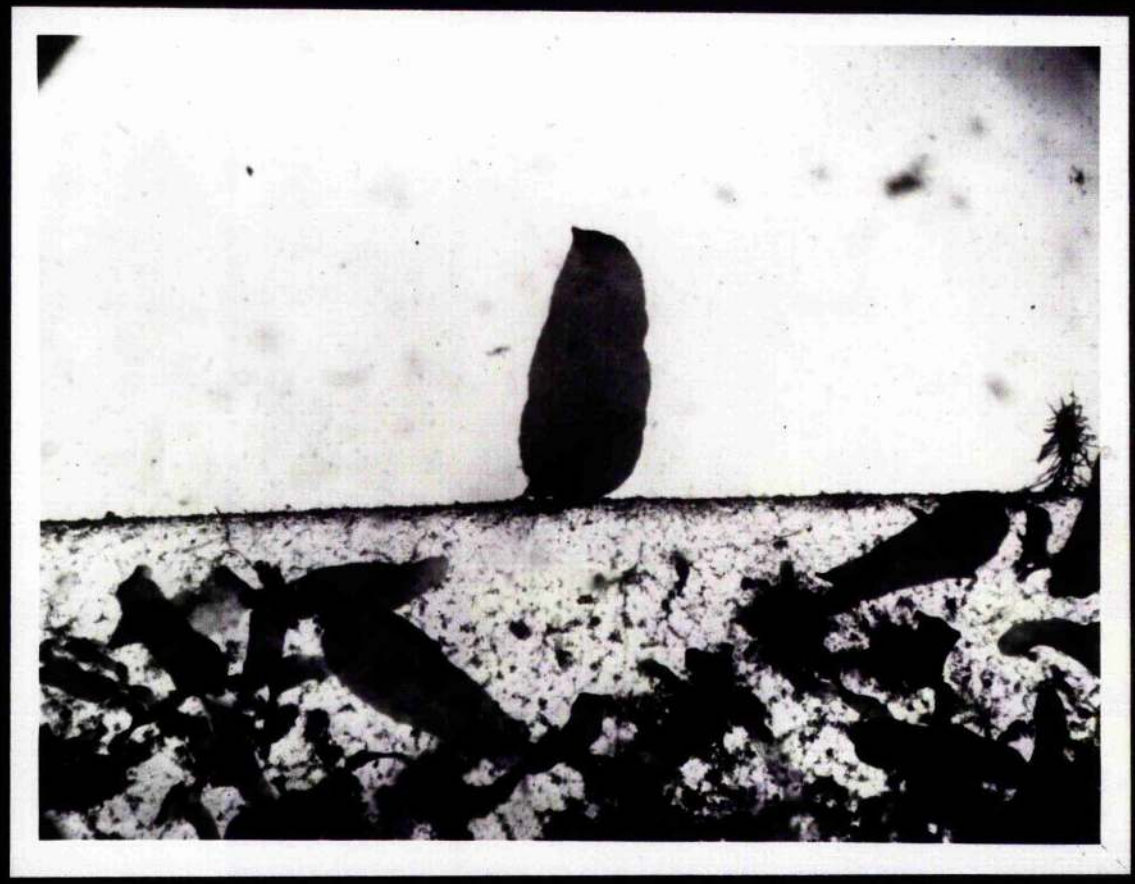
Cultured sporophytes

(a) High light culture (1.78 cal/cm^2/h)

x 6.4

(b) Low light culture (0.22 cal/cm^2/h)

x 6.4



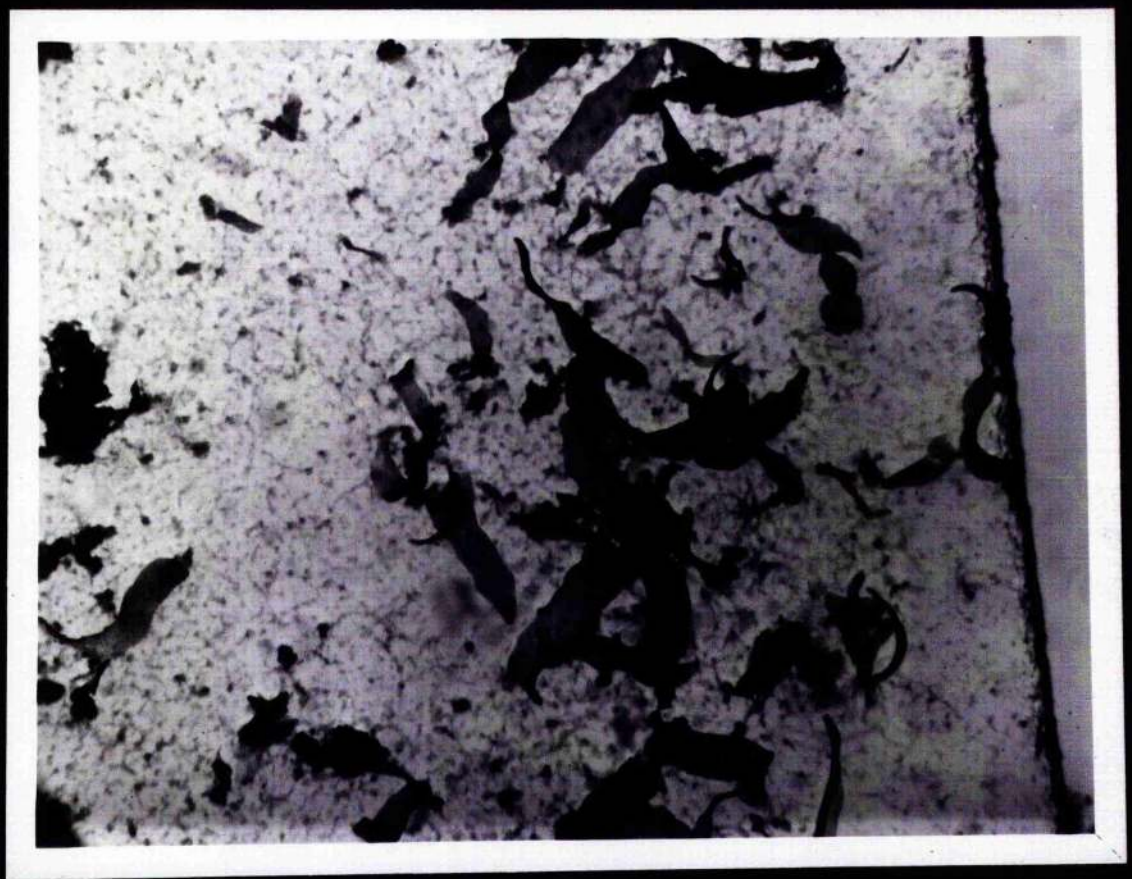


PLATE II

Electron micrograph of cultured sporophyte of L. hyperborea maintained for 66 days at 1.78 cal/cm^2/h before sectioning. Note the parallel bands of three thylakoid membranes (tm) within each chloroplast.

Details X 107,100

Ch = chloroplast
cw = cell wall
G = golgi
M = mitochondrion
N = nucleus
Nm = nuclear membrane
Pm = plasmalemma
r = ribosomes
T = tonoplast
tm = thylakoid membrane
V = vacuole

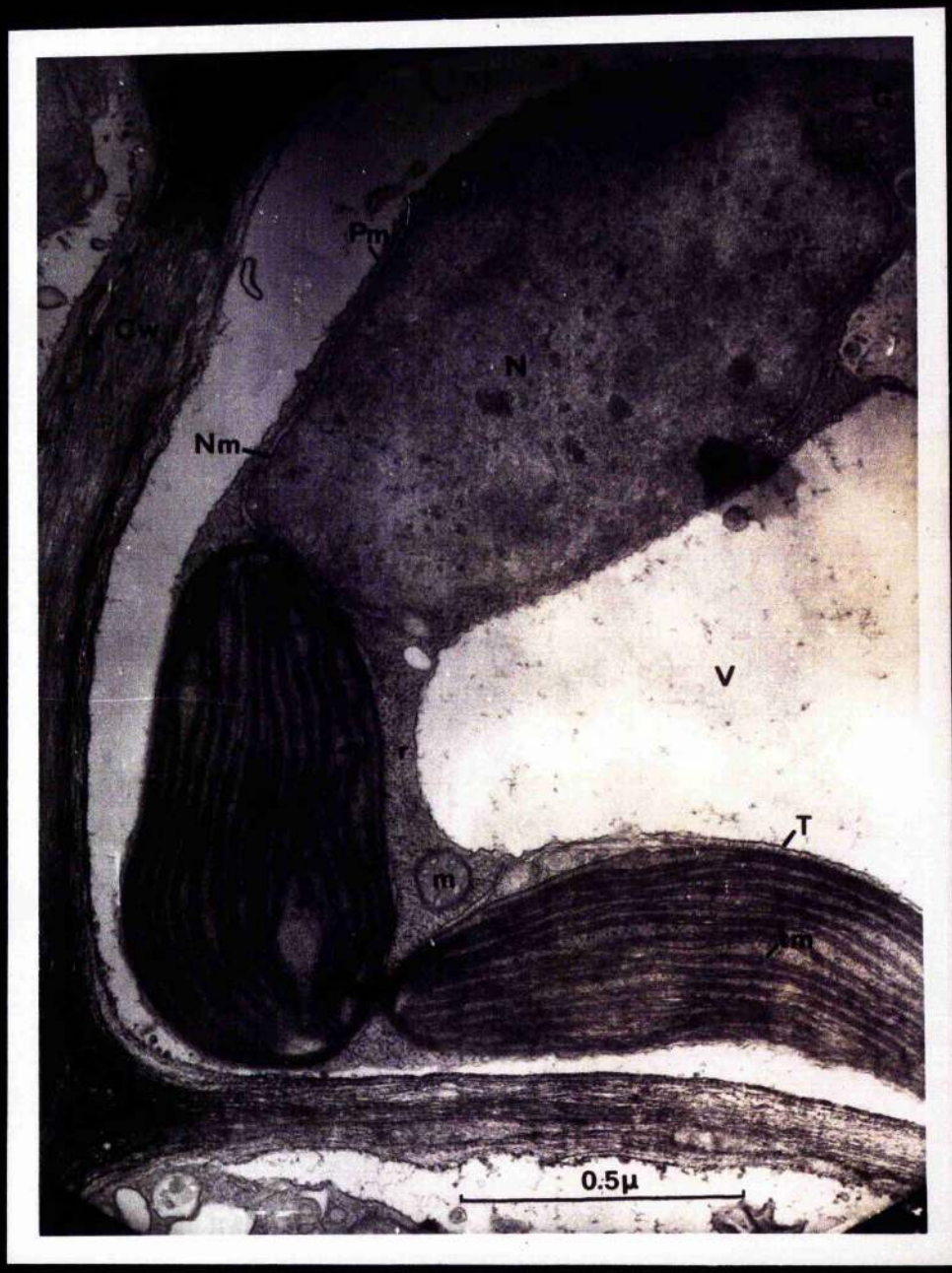

Pm
Cw
N
Nm
V
T
m
Am
0.5µ

PLATE III

Electron micrograph of cultured sporophyte of L. hyperborea maintained for 66 days at 0.22 cal/cm^2/h before sectioning. Note the absence of extra thylakoids membranes or 'stacking' between existing thylakoid bands. Note also the close proximity between the chloroplast and the mitochondrion.

Details X 171,000

m = mitochondrion

tm = thylakoid membrane

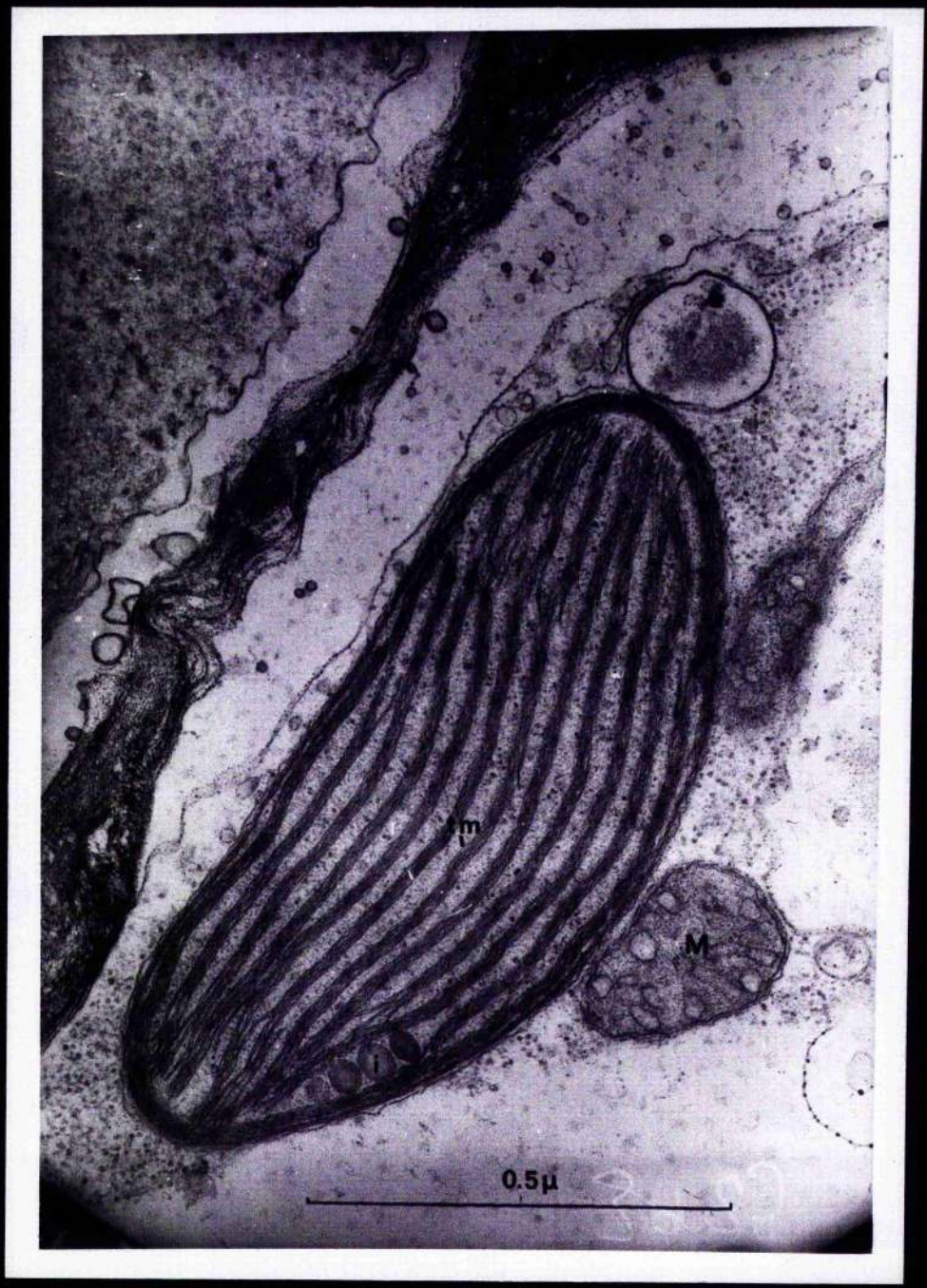

m
M
0.5μ

PLATE IV

Electron micrograph of field material of L. hyperborea. Lamina tissue sectioned 3 days after collection from 3.1m at Fife Ness. Note the large amounts of polyphenctic material (PP) in cortical cells.

Details X 21,600

C = cortical cell
Cw = cell wall
md = meristoderm cell
PP = polyphenolic material

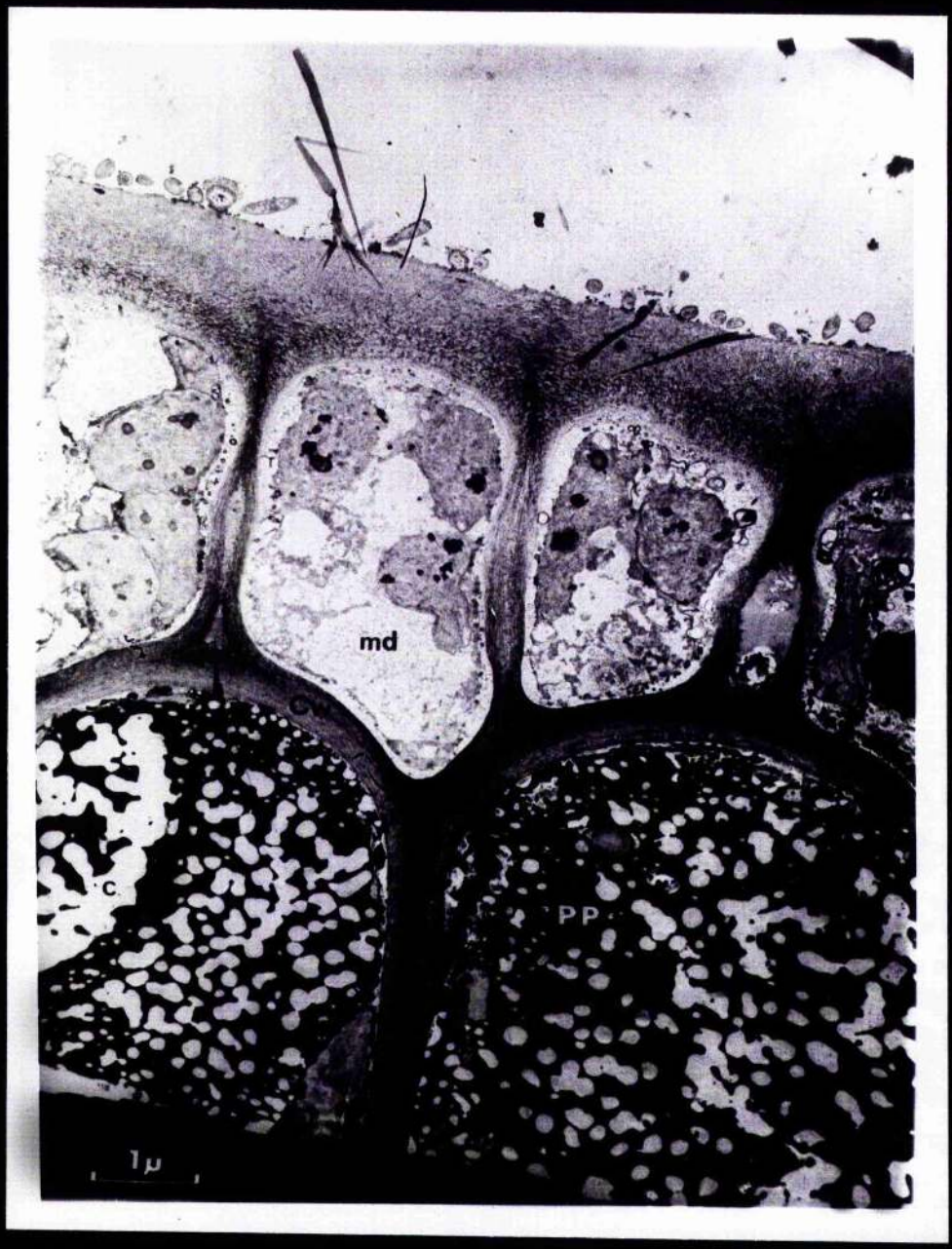

md
PP
1μ

PLATE V

Electron micrograph of field material of L. hyperborea. Lamina tissue sectioned 3 days after collection from 9.1m at Fife Ness. Note the absence of any 'stacking' of extra thylakoid membranes in the chloroplasts. Note the pit between cells.

Details X 107,100

Ch = chloroplast
l = lipid droplet
pd = plasmodesmata
pp = primary pit field
tm = thylakoid membrane

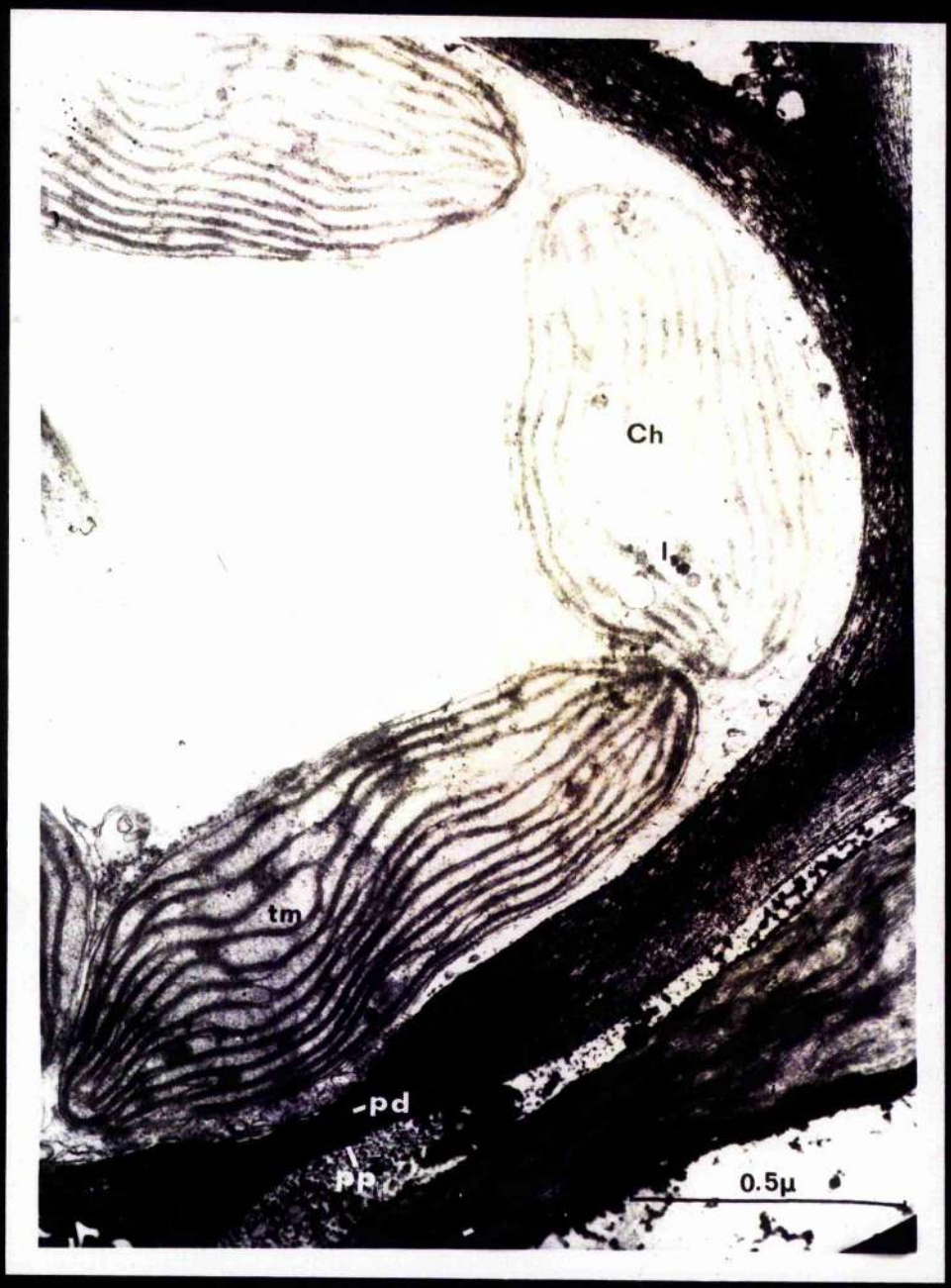
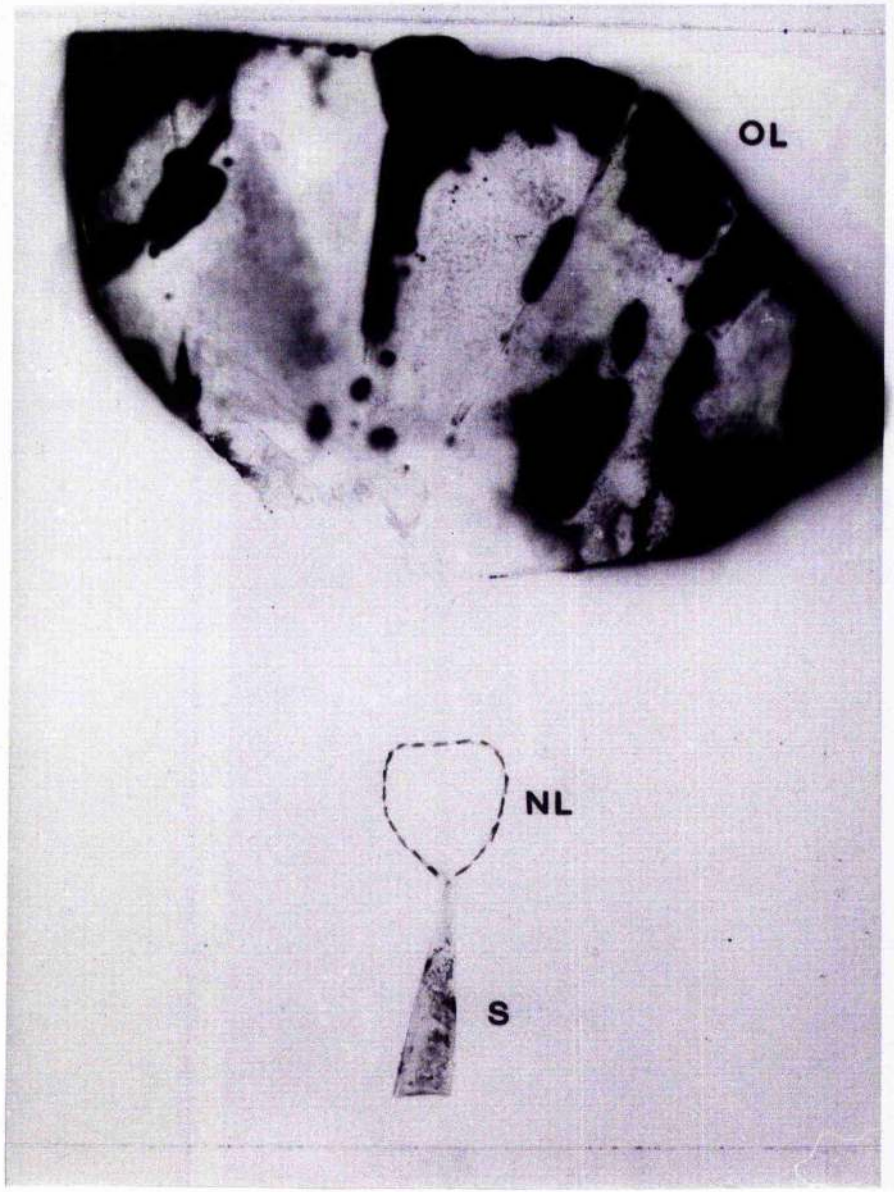

Ch
l
tm
pd
pp
0.5µ

PLATE VI

X-ray autoradiogram of translocation of C^{14} labelled photosynthate from the old lamina (OL) to the upper stipe (S). The new lamina (NL) is indicated but was not sufficiently adpressed to the film to cause fogging.



X-Ray Film of translocation from OL to S (NL)